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Biology, morphology and taxonomy of a snail-feeding leech from North Carolina, USA, provisionally identified as *Helobdella lineata* (Verrill, 1874) (Glossiphoniidae): First evidence for extra-oral digestion in the Hirudinea

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Abstract

This is a twelve-year longitudinal study of a common snail-feeding leech indigenous to the Albemarle region of northeastern North Carolina, USA. Based on contents of this paper the species is provisionally identified as *Helobdella lineata* (Verrill, 1874). For all practical purposes this is the first comprehensive description of this species. Particular attention is focused on variability of its dorsal papillae and variable pigment patterns within the Albemarle population. A total of 404 specimens were collected from 25 collecting stations in disparate parts of the region. Specialised leech traps set in these swamps were monitored regularly yielding unprecedented information on its morphology, ecology and general biology. This study recognises four principal pigment variants within the Albemarle region which, based on dissections, appear to represent a single biological species. Moreover, limited observations suggest that pigment variability is attributable primarily to adaptive camouflage to local surroundings. Methodologically it is emphasized in this paper that variable traits cannot serve as key taxonomic anchors. A proposed alternative diagnosis for identifying *H. lineata* is based entirely on more rigorous, non-variable characters. A significant finding is that *H. lineata* is most meaningfully understood in terms of specialist adaptation to feeding on snails. Furthermore, it is proposed that such adaptation required a major evolutionary shift within the foregut of this species. Evidence is presented that *H. lineata* uses uniquely large salivary cells to dissolves solid snail tissue into a semi-fluid state before ingestion via a specialised proboscis. This is the first example of extra-oral digestion in the Hirudinea.

Key words: Malacophagus, extra-oral digestion, self-fertilisation, invasive species, virgin birth, agricultural pollution, polymorphic species, longitudinal study, *Haementeria ghilianii*, *Helobdella triserialis*, leech trap

Introduction

The Albemarle-Pamlico region of northeastern North Carolina is one of the largest freshwater wetlands in the United States. This ancient swampland lies at the northern tip of a warm coastal strip where the Gulf Stream leaves the North American continent (Sawyer, 2010). An on-going ecosystematic study of the Hirudinea of this region over a period of many years documents that a species of *Helobdella* (Blanchard, 1896) characterised by several rows of dorsal papillae and variable pigmentation is indigenous to these swamps and feeds predominantly on freshwater snails. Although this snail leech is relatively common in the Albemarle region its identification is indeterminate in that it differs in detail from the several nominal ‘species’ of papillated *Helobdella* reported from North America. Designation of yet another new species of *Helobdella* is not justified on current data primarily because the Albemarle snail leech is highly variable, especially with regard to papillation, pigmentation and other taxonomically unstable characters.

The primary objective of this paper is a comprehensive description and identification of the Albemarle snail leech. Specialised leech traps set in these swamps were monitored regularly yielding unprecedented information on its general biology and ecology. Particular attention is focused on pigment variability within the Albemarle population.

This long-term study of the internal anatomy and feeding biology of *H. lineata* led to a biological overview of evolutionary significance. Namely, that feeding exclusively on snails by a proboscis leech is a highly specialised

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process which reflects a significant shift within the genus *Helobdella.* This specialisation requires unique adaptations, not the least of which is extra-oral digestion, a process unique in the Hirudinea.

The Albemarle region is a large wetland of approximately 31,000 km^2 (Fig. 1). The base of operation for the entire 12 years (2008–2019) of this study is a field station [35.719874–6.107744] located on a diked canal surrounding the entire agricultural community of Gum Neck in southern Tyrrell County, North Carolina. This facility borders the Buckridge Coastal Preserve, a sizeable game reserve outside the dike bordering the Alligator River wetlands. The dike itself was built in 1968 as a result of repeated and destructive flooding of rich farmland in the area (Sawyer, 2010, 53–55). As a consequence this dike fundamentally changed the aquatic environment of the area from clear, tea-coloured tidal water (pre–1968) to stagnant, mud-coloured water (post-1968) which now receives significant soil erosion and chemical runoff.

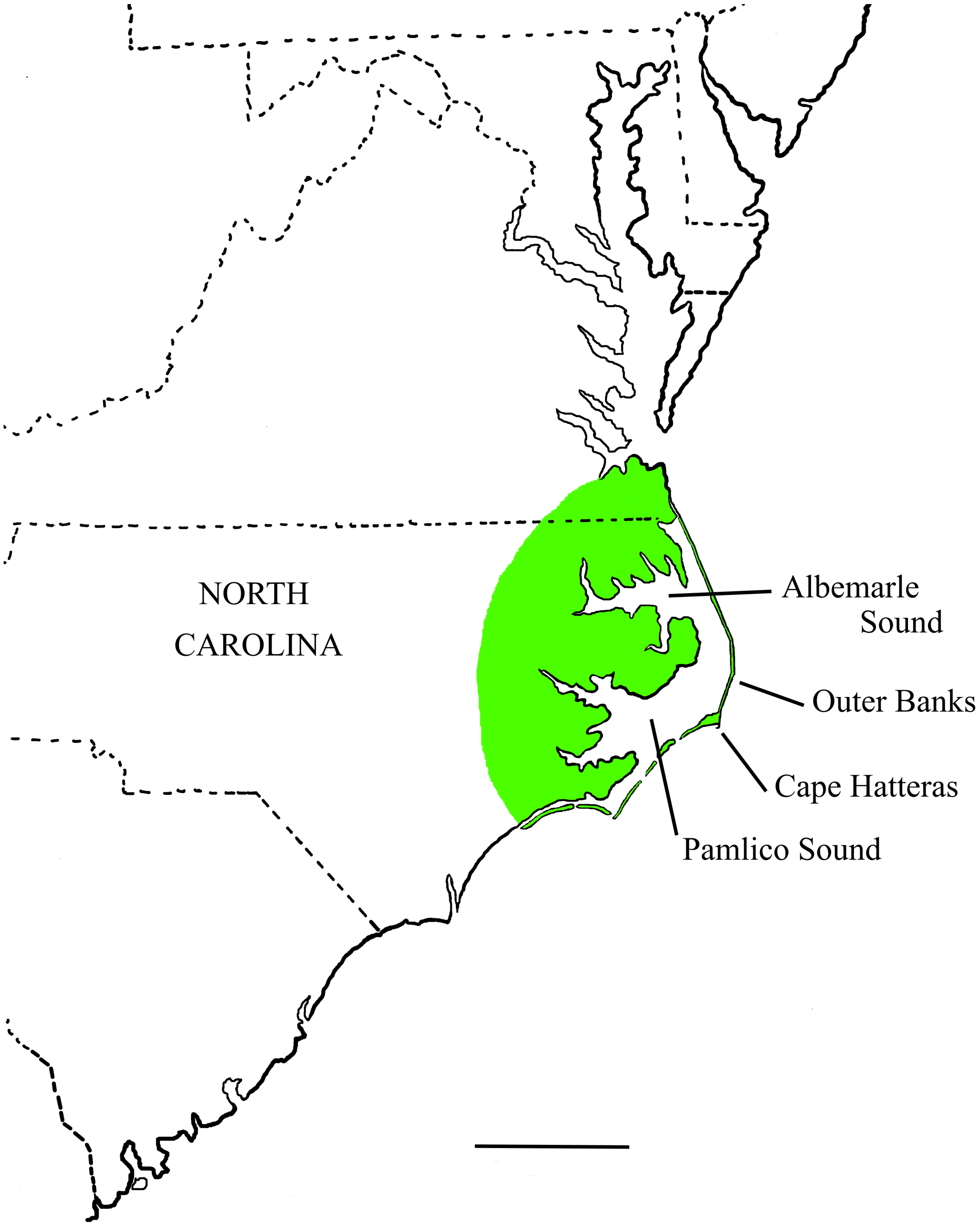

FIGURE 1. The Albemarle region of the Outer Banks in the mid-Atlantic coastal plain of North Carolina, USA. The wetlands area shaded in green is the geographic focus of this study of an indigenous snail leech provisionally identified as *Helobdella lineata*. Over 400 specimens of this heretofore unidentified species were collected over twelve years of this study. Scale bar: 100 km.

Provisional identification of the Albemarle snail leech

The Albemarle snail leech closely resembles *Helobdella lineata* (Verrill, 1874) which has been reported previously from North Carolina (Sawyer and Shelley, 1976). Unfortunately, the original description of this species is inadequate in that it is based on highly variable characters unsuitable for rigorous taxonomic identification. The current paper is the first comprehensive account of the morphology and biology of a species close to Verrill's original *lineata*. Hence, for nomenclatural stability and future taxonomic analysis the Albemarle snail leech is provisionally identified as *Helobdella lineata* (Verrill) and this name is used throughout this paper.

The description of *H. lineata* reported in this paper serves as a solid basis for determining which characters occur in all adults of this species (non-variable), and which characters do not (variable). Thus, a rigorous definition (diagnosis) of *H. lineata* summarised below can now be based entirely on taxonomically reliable characters. It must be emphasized that this species revision disregards altogether some historic but unreliable characters, notably papillation, pigmentation and crop transmutation. It is proposed that any leech which meets all criteria of the following species diagnosis is by definition provisionally identifiable as *H. lineata* (Verrill).

For all practical purposes this is the first comprehensive description of *Helobdella lineata* (Verrill, 1874). This paper is not a description of a new species, but is an account of a known species bearing the name *lineata* for 150 years.

Genus: ***Helobdella*** **Blanchard, R.1896**

Eyes 1 pair, well-separated and moveable relative to each other (Fig. 9A,B).

Species: ***Helobdella lineata*** **(Verrill, 1874) (Albemarle), Revised.**

Partial synonymy:

Clepsine papillifera var. *lineata* Verrill, 1874:683.
Helobdella lineata (Verrill, 1874): Sawyer, 1972:33–35, Sawyer & Shelley, 1976:75.
Helobdella triserialis: Sawyer, 1986:725 [not *H. triserialis* (Blanchard, E. 1849:50)]

Species diagnosis:

Feeds preferentially on freshwater snails; very large salivary cells 100 (75–165) µm, consistent with extra-oral digestion (Fig. 13A); mouth is a sub-apical slit (not an apical pore) capable of considerable enlargement when proboscis is everted (Figs 9A, 14, 15); tip of proboscis bears 14–16 stubby digitiform projections associated with salivary secretions (Figs 10D, 12); proboscis has a commodious lumen, remarkably so at its distal end, capable of receiving and transporting significant quantity of liquidised foodstuff (Figs 9A, 14, 15); the rectum is unusually conspicuous, commodious, sinusoidal and multi-chambered (Figs 7, 11).

By way of taxonomic caution, *H. lineata* (Albemarle) is indeed pigmented and typically has several rows of dorsal papillae but these are highly variable traits and not species specific (see 'Pigment variants' discussed in the narrative). Furthermore, this species typically has five pairs of crop caeca, but these are so variable and sometimes obscure or apparently absent, they are not reliable for purposes of taxonomy nor species specificity.

Parenthetically, until recently the Albemarle snail leech would have been assigned to a group defined by *H. triserialis* originally described in 1849 from Chile (Blanchard, E. 1849). However, recent molecular data disassociate *H. triserialis* (*sensu stricto*) from North America altogether (Siddall & Borda, 2003; Oceguera-Figueroa, *et al.* 2010; Kutschera *et al*. 2013). It can be argued that the elimination of *H. triserialis* (*sensu stricto*) from the North American leech fauna reinstates the taxonomic priority of *H. lineata* (Verrill, 1874, 683) in North America, reversing my earlier opinion of its taxonomic status (Sawyer, 1986, 658,725; Siddall, 2003, 31).

Methodology

Resource material Over the long period of this study a total of 404 specimens of *H. lineata* were collected from 25 collecting stations in disparate parts of the region, primarily north and south of the Albemarle Sound. Annotated locality records are compiled in the Appendix. All specimens are deposited in the Medical Leech Museum under the rubric (**HL**) or in the US National Museum (**USNM**). Reference numbers of individual leeches specifically cited in the narrative to make a point are found in the Appendix.

The current research is based entirely on specimens collected and preserved by the author in the Albemarle region. It does not include specimens from outside the region. This study focuses on adults or sub-adults, and does not encompass immature specimens.

Collecting techniques Most specimens in this long-term study were collected by means of a specially devised leech trap which proved to be integral to this study (Fig. 2). This metallic device is highly effective in attracting leeches in swamps or on muddy substrates typically inaccessible to hand-collecting. The principle behind this trap is that leeches, which move via suckers, are attracted to, and generally stay on, smooth surfaces. This device is fabricated from a sheet of aluminium folded into a rectangle (14 x 23 cm) creating a dark pouch with both internal and external surfaces. The trap is attached by a nylon cord which allows the trap to be thrown to a specific position in the water and monitored overnight. In this study the same traps have been used in the same locality for up to the 12 years, a veritable longitudinal study. From time to time it is advisable to clean the surface of the trap to eliminate mud and algae. Similarly, the traps should be soaked occasionally in bleach and thoroughly rinsed to eliminate interlopers, as appropriate. On other occasions hand collecting with waders to check vegetation, cans, bottles, plastic, turtles, and even the waders themselves is productive. For pragmatic reasons most field work was carried out each year from March to June, especially the month of May, the peak of the breeding season. No field work was carried out in winter (December–February). Most specimens were collected in southern Tyrrell and Pasquotank Counties, but every effort was made to collect as widely as practical (see Appendix).

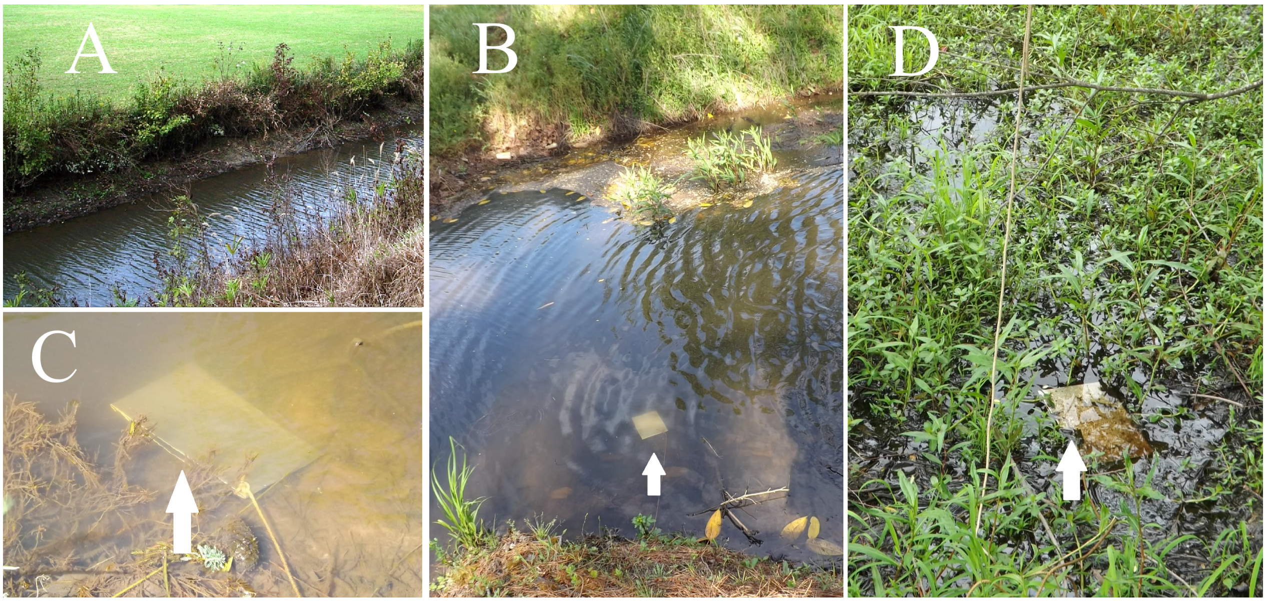

FIGURE 2. Two types of aquatic habitat in southern Tyrrell County contain snail leeches provisionally identified as *Helobdella lineata* with different respective pigment patterns, suggesting pigmentation in this species is an adaptation to surroundings rather than inherently taxonomic. A–C, Agricultural dike canal draining low farmland [35.719874–76.107744]. Virtually all leeches collected in this muddy canal have typical metameric white spots, described in the paper as pigment variant #1. D, Sandy shoreline of a swamp with typical lush vegetation and clear water [35.721466–76.10195]. All individuals at this site have longitudinal stripes (described herein as pigment variant #4), i.e., lacking metameric white spots. In spite of differences in pigmentation the two respective habitats are only 0.55 km apart. The canal (A–C) is location of the 12-year population study presented in this paper. Arrows, aluminium leech traps (roughly 14 x 23 cm).

Materials and methods

Other species. Extensive collecting documents that three species of *Helobdella* co-exist in the Albemarle region (Appendix) (Fig. 3), namely *H. stagnalis, H. elongata* and *H. lineata*. *Helobdella stagnalis (sensu lato*) as used in this paper is understood to mean *H. stagnalis* clade which also encompasses *H. modesta* (Verrill, 1872) as currently understood (Moser *et al.* 2011), and *H. octatestisaca* Lai, Chang and Chen, 2009. For practical purposes *H. stagnalis (sensu lato*) is treated as a single species.

The two sympatric congeners, *H. stagnalis* and *H. elongata,* are taxonomically outside the snail-feeding clade (Siddall & Borda, 2003) and are hereby recognised as instructive species for taxonomic comparison and reference. A total of 198 specimens of *H. stagnalis* were collected in this study and stored in the Medical Leech Museum collection under the rubric (**Hst**). Only 3 specimens of *H. elongata* were collected during this study and stored under the rubric (**HE**). Comparisons with *H. lineata* have relevance to the broader question of adaptive evolution within the genus *Helobdella* (see Discussion). Occasionally other nominal 'species' of *Helobdella* from elsewhere are discussed to make a particular point.

Arguably, the feeding apparatus of *H. lineata* is analogous to the proboscis of blood-feeding glossiphoniids. In this context the Amazon leech *Haementeria ghilianii* is of particular relevance in that this species is closely allied to the genus *Helobdella* which importantly is thought to have a centre of species distribution in South America (Siddall & Borda, 2003). For salient taxonomic features of *H. ghilianii* see Sawyer (1986, 36–39, 65, 302–3, 479–90, 633–4, 654). Accordingly *H. ghilianii* is recognised as an especially useful species for comparing the adaptive evolution of the specialist malacophagus foregut of *H. lineata* versus mammal- and reptile-feeding *H. ghilianii.* Occasional reference is made to the sympatric blood-feeding turtle leech *Placobdella multilineata*.

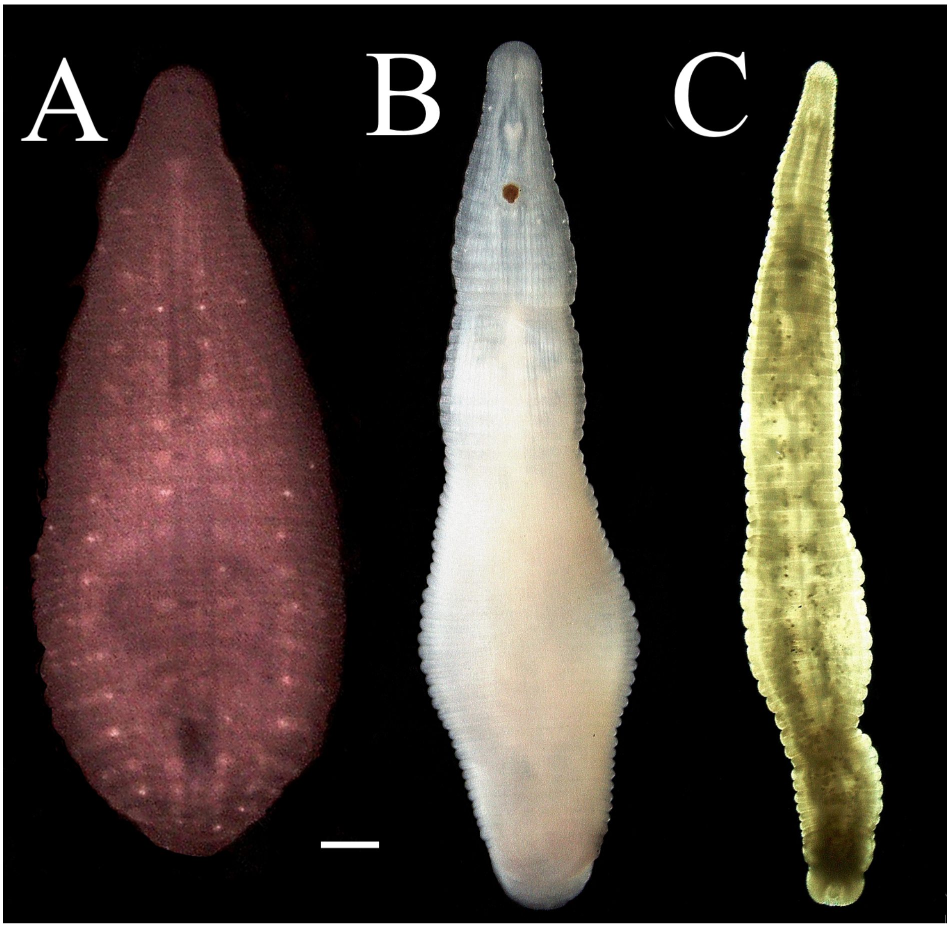

FIGURE 3. Three co-existing species of *Helobdella* indigenous to the Albemarle region of the Outer Banks, North Carolina. A, *H. lineata*, the subject of this paper, photographed in life, pigment and papillar pattern intact (HL-26); B, *H. stagnalis* (*sensu lato*) (Hst-1A); C, *H. elongata* (HE-2A). Scale bar: 1.0 mm.

Methodology of species identification As a fundamental taxonomic principal in this paper it cannot be over-emphasized that variable traits cannot serve as key taxonomic anchors and accordingly are not used herein to define *H. lineata*. In this context identification of this species is based rigorously on non-variable traits, i.e. those which occur in all adult individuals of this species. Conversely, species diagnosis explicitly excludes those traits which do not occur in all adult individuals.

The Albemarle population of snail leeches is represented by several distinct pigment variants of undetermined taxonomic status. A primary question asked in this paper is whether these variants represent several respective 'species' or one biological taxon which lives throughout the Albemarle region? A follow-up question is which characters are non-variable and therefore taxonomically reliable for purposes of identification? Such stable traits contribute disproportionately to the taxonomic revision proposed in the Introduction. Throughout this study variability of external features is based primarily on living specimens examined microscopically. Variability of internal features is based on dissections of preserved specimens, as well as on micro-photography through the unusually translucent body walls of most preserved specimens in this collection.

Archetype specimen. In the eventuality that future studies demonstrate that the Albemarle snail leech indigenous to this region may constitute more than one biological species a single adult specimen (Fig. 4) is selected as representative of *H. lineata* described in this paper. The external and internal features of this type specimen serve as a reference or baseline against which comparisons can be made, especially with regard to variability.

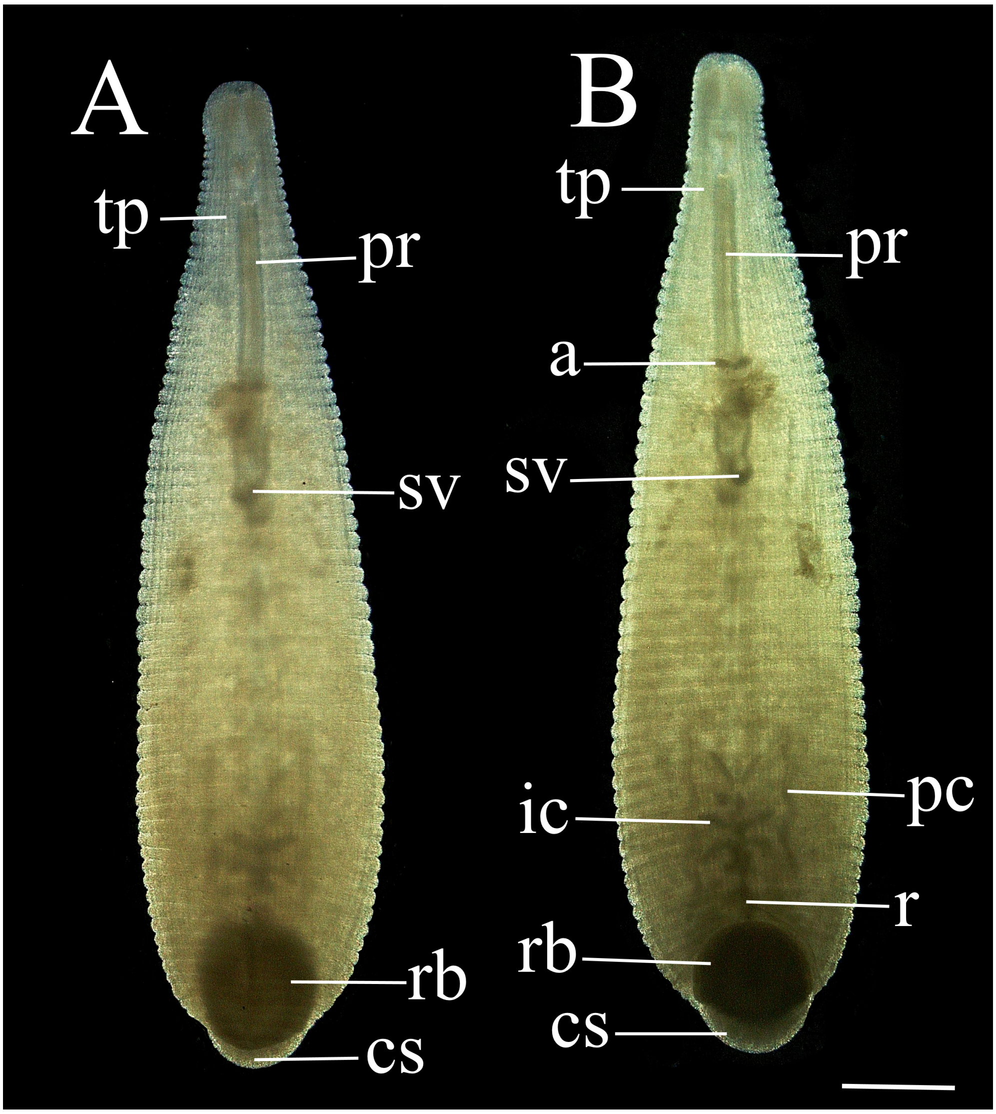

FIGURE 4. The archetype or reference specimen (HL-5A) on which the description of *H. lineata* is based. This specimen has faded and become translucent following preservation with 5% formalin. The type locality is in Pasquotank County, NC [36.191176, -76.228516] A, dorsal view. B, ventral view. Abbreviations: a, atrium; cs, caudal sucker; ic, intestinal caecum; pc, post caecum; pr, proboscis; r, rectum; rb, rectal bladder; sv, seminal vesicle; tp, tip of proboscis. Scale bar: 1.0 mm.

Segmentation and annulation The term 'sXIII ' or simply 'XIII' refers to segment thirteen written in Roman numerals, whereas 'gXV' refers to ganglion of its respective segment fifteen. Each mid-body segment is comprised of three encircling annuli (rings) written from anterior to posterior as a1, a2 and a3, respectively. The designation XIa3/XIIa1 refers to the furrow between the third annulus of segment XI and the first annulus of segment XII. This is the location of the male gonopore and is an invariant landmark at the ventral midline of this species. The more obscure female gonopore is positioned one annulus to the posterior, i.e. at XIIa1/a2, i.e. the furrow between the first annulus of segment XII and the second annulus of segment XII.

Terminology The term 'segment' is used in preference to 'somite' throughout this paper. The former is used traditionally for anatomical landmarks in the Hirudinea generally, whereas the latter is more common in an embryological context.

The term 'papilla' is used herein in preference to 'tubercle', without comment on any anatomical or functional distinction. However, in general, papilla is a small dark-tipped structure, as typically found on the dorsum of *H. lineata*, whereas tubercle is a larger and more conspicuous structure, as typically found on the dorsum of turtle leeches *Placobdella multilineata* in this region (pers. obs.).

The term 'eye' is used in preference to 'eyespot'. In this paper the former more clearly conveys anatomical structure or integrity than 'eyespot'.

In the context of this paper 'extra-oral digestion' refers to enzymatic digestion of prey tissue into a liquid state prior to being ingested via the proboscis of a snail-feeding leech.

Preservation and dissection Prior to preservation the specimen is relaxed by adding a few drops of alcohol at intervals until there is no movement even under hot water. After removing mucus with a damp paper towel the specimen is laid out linearly in a dissecting dish without stretching, lightly pinned if necessary, and then slowly flooded with 5% formalin. After several hours the specimen can be stored in 5% formalin in preference to 10% formalin which makes the tissue too brittle for dissection or 70 % alcohol which tends to dissolve pigment. Preserved specimens should be stored in darkness because of a tendency of this specimens to lose most, if not all, of its pigmentation over time. As a safeguard, specimens should be photographed in life prior to preservation.

Five adult individuals (10.5–18.5 mm) from disparate parts of the Albemarle region were dissected. These represented Hertford Co. (HL-13A), Pasquotank Co. (HL-8A; HL-5A), Tyrrell Co. (HL-15A) and Wayne Co. (HL-6A) (Fig. 5). Each individual to be dissected is placed dorsal side up in a wax-bottomed tray and flooded with dechlorinated water. Because of the relatively small size of this species strategic positioning of insect pins is important. It is advisable initially to secure an insect pin on either side of the posterior end of the body, but not through the caudal sucker. The anterior end of the individual is secured by overlapping two insect pins at about level of the 'neck' region, not through the tissue itself so as not to destroy the fragile proboscis complex. An initial transverse incision is made mid-dorsally about mid-body. A shallow incision using fine scissors follows an annular furrow to the lateral margins on either side. Another incision is made anteriorly at the respective margins. The tegument is very carefully teased to expose underlying features. The crop caeca and reproductive structures are exceptionally delicate and therefore difficult to maintain integrity. Accordingly, digital photographs should be taken at appropriate stages for future reference. Printed images constitute useful outlines for line drawings.

Translucent body wall: visualisation of internal anatomy An unusual, if not unique, aspect of this study is that most specimens of *H. lineata* examined in this study had faded significantly over time due to the instability of body pigment (Fig. 4). As a consequence of losing most of their body wall pigment the specimens are remarkably translucent. Thus, it is possible in many cases to clearly visualise three-dimensional images of internal anatomy without destroying the specimen, not unlike 3-D tomography (for example, see figs 7B, 15C).

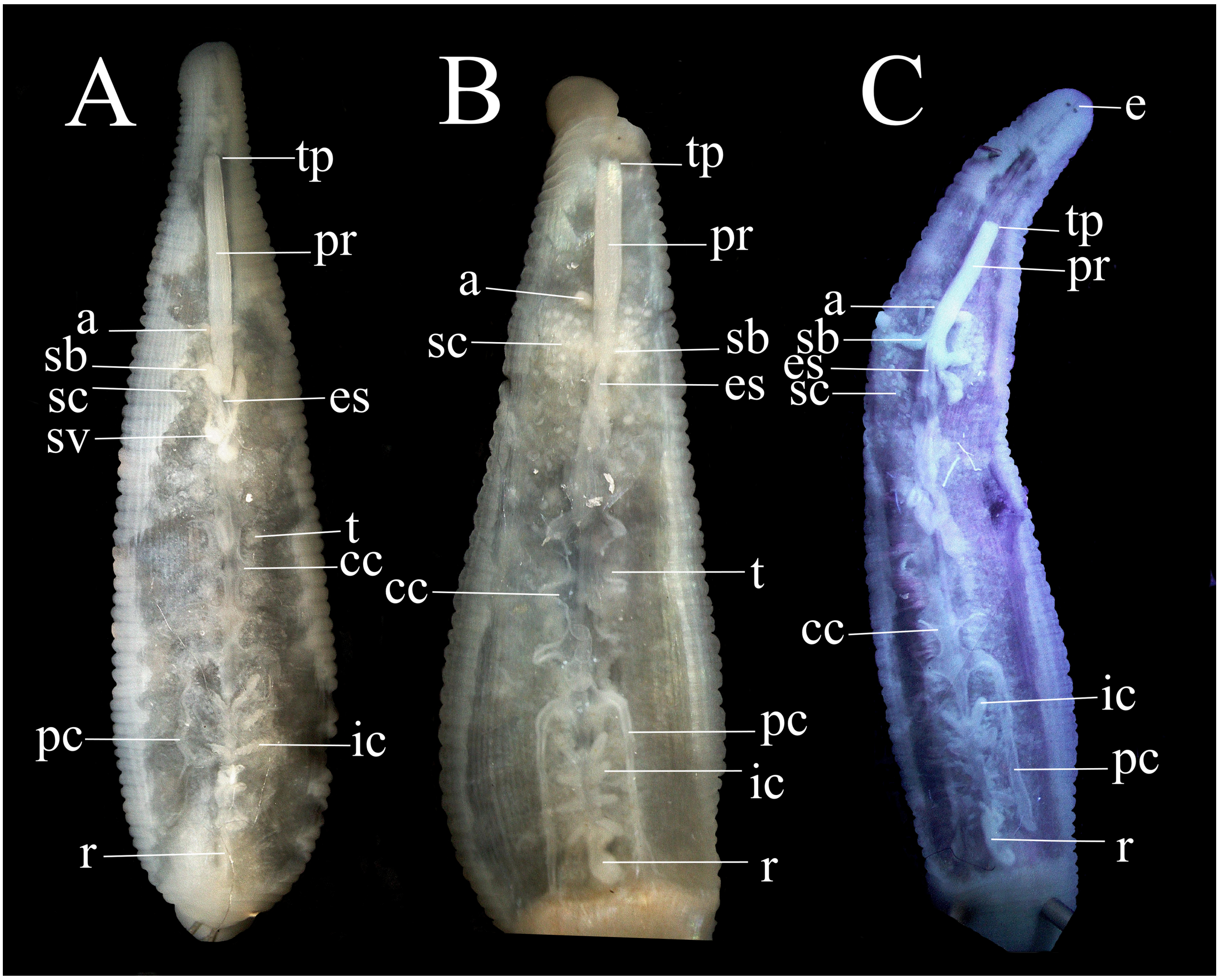

FIGURE 5. Dissections of individuals of *H. lineata* from disparate locations in the Albemarle region reveal that certain features of the digestive and reproductive systems are anatomically invariant. This relative lack of internal variability corroborates the tentative view they all represent the same biological species in this region regardless of pigmentation and papillation. A, Pasquotank County (HL-5A, 12.8 mm); B, Wayne County (HL-6A, 18.5 mm); C, Tyrrell County (HL-15A. 15.5 mm). Abbreviations: a, atrium; cc, crop caecum; es, esophagus; ic, intestinal caecum; pc, posterior crop caecum; pr, proboscis; r, rectum; sc, salivary cell; sb, salivary bundle; sv, seminal vesicle; t, testis; tp, tip of proboscis.

Counterstaining In view of the translucence of many faded specimens it is sometimes useful to counterstain with two drops of a 2% solution of methylene blue into 5 ml of water for several hours. This technique is especially practical for highlighting salivary cells and testes.

Microscopy Microphotography constitutes a substantial part of this study. Most individuals were photographed dorsally and ventrally under various magnifications and lighting. Each specimen is uniquely labelled corresponding to the reference number (**HL**) in the Appendix. This visual catalogue of virtually the entire collection was performed by means of a Wild M7A stereo microscope with a Volpi Intralux 4000 light source, complemented by a Lapsun Spot Point LED lamp and ultraviolet facility. Close-up images were taken with cameras of increasing magnification: 1) Conrad Electrics, Mirazoom MZ902, DP-M14; 2) Yuanj MC500 eyepiece camera for stereo microscope; and 3) Bresser LCD 5 MP microscope with slide stage and photomicrograph facility. Measurements were taken with Ocular Micrometer Model WF 10X.

Results

Population study of pigment variants of *H. lineata*

Snail leeches of the Albemarle region are highly variable and from the outset of this study biologically difficult to interpret. For example, how many valid species are represented in this region, and/ or whether adaptive camouflage is the significant factor in sustaining such pigment variability across the region? In the first *Helobdella* population study of its kind hundreds of *Helobdella* snail leeches from disparate parts of the region were individually examined alive to gain insight into these and related questions prerequisite to the course of this study. The tentative conclusion from these observations is that, in spite of considerable variation, morphological evidence indicates that only one species of snail-feeding *Helobdella* is indigenous to the Albemarle region. Furthermore, analysis of separate specific populations indicates that individuals are highly adapted to their local surroundings, be it light sand or dark mud.

Of the more than 400 specimens examined in this study, pigment variants could be categorised into four main groups, at least three of which clearly represent a single biological taxon upon dissection.

Pigment variant #1 (97.8% of collected specimens). This variant #1 is overwhelmingly the most abundant form of snail leech encountered in this study, bearing in mind that even within this group there is further, but minor, variability. Owing to its predominance the morphology of pigment variant #1 is the primary basis of taxonomic conclusions expressed in this paper. For convenience, pigment variant #1 is recognised as the standard *H. lineata* and is tagged accordingly throughout this paper.

Variant #1 is characterised by two pairs of dorsal, longitudinal metameric white spots on either side of a darkish median strip (Fig. 6A). As a rule the intermediate and paramedial spots, respectively, are positioned on the middle annulus (a2) of each segment. Each white spot is relatively large in that it generally covers the width of its respective annulus. Those in the paramedial rows are the more conspicuous and extend anteriorly to approximately segment VIII. In this variant the white spots are void of pigment rather than possessing white pigment.

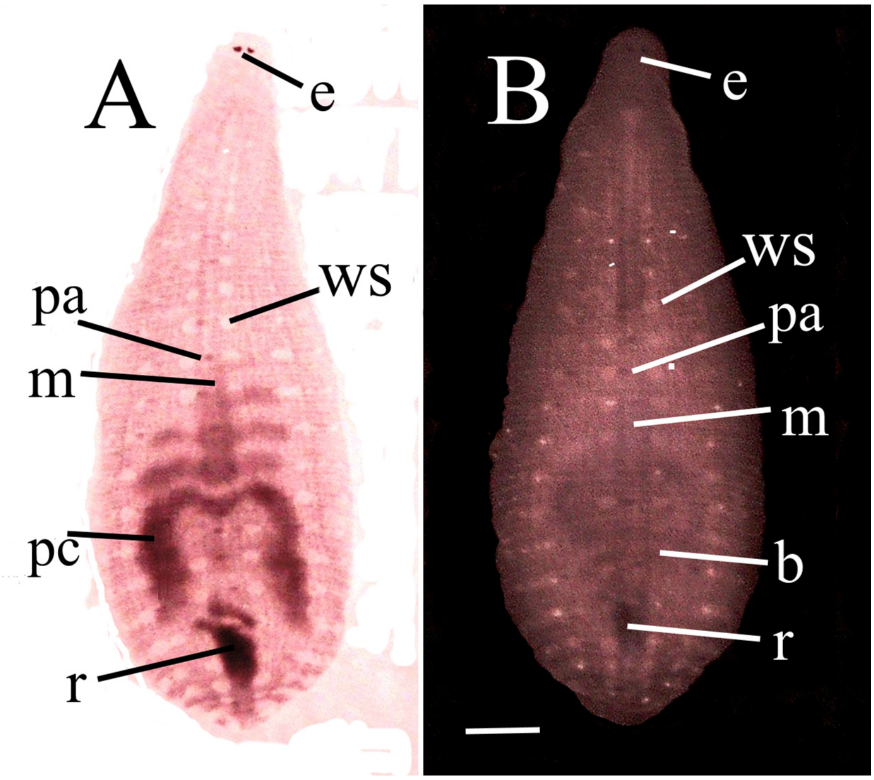

FIGURE 6. Variation in dorsal pigmentation and papillation in *H. lineata*. Two individuals collected from the same location. A, (HL-4), lightly pigmented with metameric spots. B, (HL-26), darkly pigmented with metameric spots. By far the majority of individuals throughout the Albemarle region were lightly pigmented with metameric white dots as illustrated in A. Note: pigmentation disappeared quickly after preservation making the specimen translucent. Abbreviations: b, brooding depression on ventral side; e, eye; m, medial stripe; p, posterior crop caecum; rectum; pa, papilla; ws, metameric white spot. Scale bar: 1.0 mm

In addition to metameric white spots the dorsum of pigment variant #1 typically bears three longitudinal rows of scraggy and irregular papillae primarily on the a2 annulus in the dorsal midline and intermediate locations, respectively. Papillae are most prominent in the medial, mid-dorsal row. The papillae of variant #1 are relatively small and inconspicuous, and are sometimes apparently absent for all practical purposes. Papillae are distinguished primarily by their relatively darker coloration against the lighter background.

The ground colour of variant #1 is a homogeneous light reddish brown whose pigment lies in barely discernible longitudinal threads. Variant #1 varies somewhat individually depending on how heavily pigmented is the ground colour. Variant #1 resembles *H. lineata* as described in Sawyer (1972, 34).

Pigment variant #2 (1.0%). This dark cryptic variant has an almost overpowering dark ground colour whose pigment somewhat obscures the presence of the underlying metameric white spots, median strip, and papillae (Fig. 6B). Most significantly many of these 'white' spots have a very prominent cluster of white, almost lustrous cells (chromatophores) at their centre. The specimen shown in Fig. 6B is remarkable in that it displays metameric white spots (white owing to absence of pigmentation) in the middle of which is a cluster of chromatophores (white owing to a cluster of almost lustrous cells, rather than absence of pigment). Interestingly, these two ways of forming white spots (unpigmented area vs white chromatophores) have been shown in *H. robusta*, another 'variant' of *Helobdella* (Shankland *et al*. 1992, fig. 2B). The function of this lustrous white metameric chromatophore in the dermis of pigment variant #2 is incompletely known (Blair, 1993).

Pigment variant #3 (0.25%). This peculiarly mottled pattern was observed in only one individual (10.5 mm), in Hertford County (HL-13). At the time of collection it was described in the author's field notes as 'erratic, similar to the pattern of *Placobdella parasitica*'. An image from life was not possible due to rapid fading of pigment upon preservation. This individual somewhat resembles that illustrated elsewhere (Sawyer, 1972, fig. 4E; Siddall & Borda, 2003, fig. 3H). This individual (HL-13) was dissected and appears to be virtually the same internally as other dissected individuals in this study (Fig. 5).

Pigment variant #4 (1.7%). This longitudinally-striped variant #4 is uncommon but easily distinguishable. It is noticeably different from the other variants collected in the Albemarle region in that its dorsum has longitudinal white stripes instead of metameric white spots. Only seven adult specimens of the longitudinal variant were collected in the Albemarle region during this study. In one locality (HL-1) in Tyrrell County all six specimens collected at this site had this distinctive pigment pattern. Whereas, variant #4 was the only variant collected at this site, a roadside vegetated swamp (Fig. 2D), it was located only 0.55 km from a muddy drainage ditch in which virtually all specimens were variant #1 (Fig. 2A, B, C). This observation suggests the pigment differences between #1 and #4, respectively, may reflect adaptation to surroundings, rather than species differences.

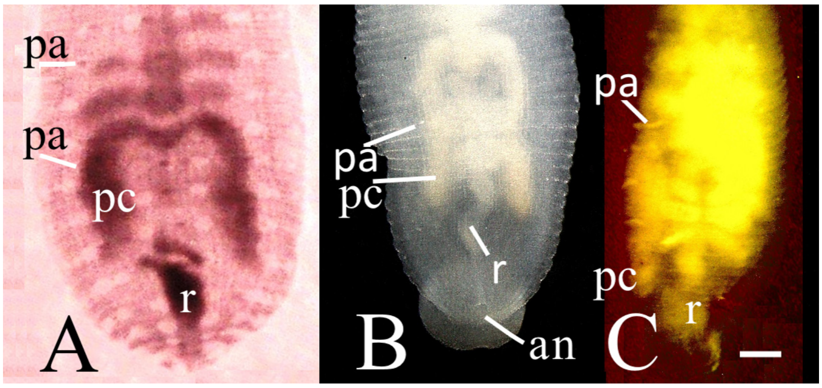

FIGURE 7. Caudal end of *H. lineata* showing arrangement of dorsal papillae and the rectum under different conditions of lighting and preservation (5% formalin). A (HL-4), in life, under natural light. B, faded preserved specimen (HL-9B), LED light. C, faded preserved specimen (HL-9F), LED light. PAPILLAE. A, papillae are dark and conspicuous, located on a2 (middle) annulus along the medial stripe and the intermediate position. B, papillae are present but unpigmented (i.e. faded) and barely discernible. C, peculiar columnar link between external papillae and the underlying crop caeca. RECTUM. A, brown foodstuff visible in the crop caeca. Note: the rectum consists of two adjacent compartments. B, the rectum is visible through the translucent body wall as a slightly convoluted tube leading to a relatively large anus. C, the rectum has an apparent anterior third chamber located behind the intestine. Abbreviations: an, anus; pa, papilla; pc, posterior crop caecum; r, rectum. Scale bar: 1.0 mm.

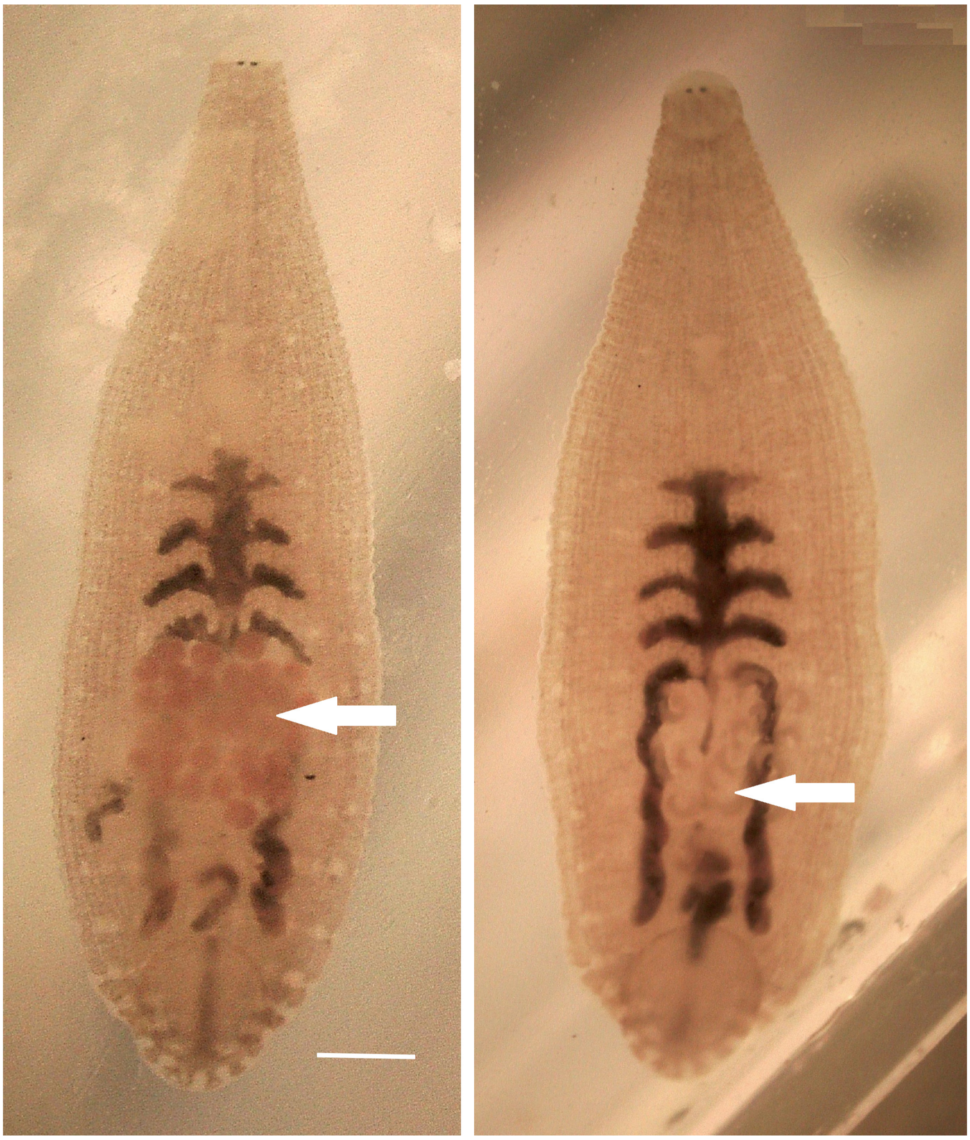

FIGURE 8. Views of the same individual of *H. lineata* (HL-26) reared in isolation in UK and gave rise to viable offspring on three occasions without intervening fertilisations. Batch 1(left): egg sacs had been laid on venter on 8 Jan, and photographed five days later prior to hatching (arrow). Batch 2 (right): same individual without subsequent fertilisation deposited a second batch of eggs on 18 Feb; viable embryos (arrow) were photographed 11 days later. Note five distinct pairs of crop caeca in both images but the configurations of the caeca are markedly different. Batch 3 (not photographed) was laid on 28 March. Scale bar: 1.0 mm.

That longitudinally-striped individuals do not represent a distinct species is corroborated by another observation, in which a single individual of variant #4 was collected along the rim of a mill pond in Wayne County (HL-7). In exactly the same locality (HL-6) four individuals of variant #1were also collected, precisely a year earlier. Dissection of one of these individuals (HL-6A) confirmed it was same biological taxon as the Albemarle snail leech (variant #1) described in detail in this paper (Fig. 5B).

Furthermore, it has been shown by others in a laboratory study that a normally striped 'species', *H. robusta*, reared in isolation for ten generations did not always breed true in that fewer than 1% of the offspring were metamerically spotted, rather than striped (Shankland *et al.* 1992).

An image of the longitudinal variant #4 from life was not possible because its pigment disappeared soon after preservation. Nonetheless, these records of variant #4 are reminiscent of the pattern illustrated elsewhere (Sawyer, 1972, fig. 4D; Siddall & Borda 2003, fig. 3E).

Adaptive significance of pigmentation (camouflage) In addition to the foregoing section, other evidence in this study suggests that variability of pigmentation in this leech reflects adaptation to surroundings. For example, in one specific sandy pond (HL-22) its leeches were much lighter than those collected the same day from a nearby muddy drainage canal (HL- 23). Moreover, most collecting sites had only one predominant pigment pattern, but on occasion a second pattern may also be present in the same or nearby locality (Fig. 6). In the abovementioned sandy pond, one darkly pigmented individual was found amongst otherwise light-coloured individuals.

Instability of pigment Throughout this study it was observed repeatedly that pigmentation of *H. lineata* is unstable. Virtually all preserved specimens faded relatively quickly. Even in living specimens a segmental portion may be unpigmented independently of the rest of the body. For example, in one living specimen (HL-26) the tip of the head completely lacked pigment anterior to the eyes, the sharp line of demarcation being at IIIa1/(a2+a3) (Fig. 8, left). The significance of this remarkable demarcation is unclear.

The instability of pigment in *H. lineata*, along with known 'colourless' forms outside this geographic region (Sawyer, 1972, fig. 4F; Siddall & Borda, 2003) suggests that pigmentation per se is an unreliable taxonomic character.

Papillae The biological significance of dorsal papillae is not known but may be linked functionally to the gut. For example, *H. lineata* appears to have an anatomical bridge between the external papillae and the underlying crop (Fig. 7). The function of this peculiar link between the dorsal tegument and the gut is unknown. However, the functional significance may be considerable in that a similar link occurs in the heavily tuberculated sympatric species *P. multilineata* (pers. obs.).

Description of *Helobdella lineata*

External features

A single adult specimen (HL-5A) is selected as being representative of the snail leech indigenous to the Albemarle region (Fig. 4). The following description of this relatively large individual (12.8 mm) (representing pigment variant #1) is supplemented by observations on eleven additional specimens collected at the same location (HL-5) in Pasquotank County. These twelve cohorts are remarkably homogeneous in size (9.8–12.8 mm), and state of feeding (empty gut) and reproduction (not gravid). External variability is based on examination of the additional 392 specimens of this species collected throughout the Albemarle region during this study.

Typical of *H. lineata*, all twelve individuals in the archetype series had quickly faded in the preservative with the consequence that pigmentation is generally lost. The pigment pattern described in this section is based primarily on additional specimens photographed in life (HL-24, HL-26)(Fig. 6) or recorded at time of capture.

Size Inexplicably, the three largest individuals of *H. lineata* (18.5, 16.5 and 15.5 mm, respectively) collected in the 12 years of this study came from precisely the same locality, namely Williams Mill Pond in Wayne County (HL-6). In contrast, the leeches collected in the rest of the Albemarle region were significantly smaller (mean, 7.2 mm; range, 2.2–12.5 mm; N = 63), with exception of one large individual (15.5 mm) collected in a diked canal in Tyrrell County (HL-15A).

Body shape Living individuals of *H. lineata* are generally flat. At rest they are widest in the posterior third of the body, and the head and oral sucker are somewhat rounded, not pointed. Interestingly, preserved individuals change body shape, becoming noticeably more narrow and rounded after preservation.

The reference specimen (HL-5A) is straight (12.8 mm) and well preserved but its pigment has faded entirely (Fig. 4). Consequentially, the proboscis (length, 2.8 mm) and atrium are prominent landmarks visible through the translucent body wall. The maximum body width (3.3 mm) is located toward the posterior end of the body at about segment XIX. From this point the body narrows anteriorly slightly to about segment XI. From here the body narrows increasingly to the oral sucker (width, 0.8 mm). The distance from the atrium (male gonopore) to the tip of the oral sucker is 3.8 mm, and the width of the body at the male gonopore is 2.1 mm.

Gravid and brooding individuals are recognisable because the lateral margins of the body curve downward and inward to form a slight protective cavity. This swells the line of the mid-body.

Oral sucker and mouth Dorsally the oral sucker is almost indistinguishable from the rounded head. From a ventral perspective, however, the oral sucker is clearly outlined as a broad triangular rim (0.5 x 0.9 mm).

The mouth of *H. lineata* differs from most other glossiphoniids in being a conspicuous slit located noticeably closer to the centre of the oral sucker than to the sucker apex. This peculiar anatomy probably reflects the unusual feeding habits of this snail-feeding leech. In this context, it is proposed that the open slit functions as a distensible jaw, capable of receiving the relatively large proboscis when the latter is everted (Fig. 12A). In fact, as discussed elsewhere, the proboscis can expand further upon imbibing fluid from a pre-digested snail. Structure of the mouth-proboscis complex of this species is of considerable taxonomic significance and contrasts with the small pore located very near the apex of the oral sucker as found in the sanguivorous allied species, *H. ghilianii* (pers. obs.).

Mouth-like slits occur in virtually all specimens of this species collected in the Albemarle region and is considered a non-variable character for taxonomic purposes. Most individuals have closed slits, but the mouth of the reference specimen (HL-5A) is slightly open to reveal a puckered inner lining. Open slits are found in a few other individuals (for example, HL-13A, HL-15A).

<u>Eyes</u> As a rule *H. lineata* has two and only two well-separated eyes (Fig. 9A,B). This is also the case for the sympatric congener species *H. stagnalis* and *H. elongata*, and is considered characteristic of the genera *Helobdella* (Blanchard, R. 1896) and *Haementeria* (Filippi, 1849) (Sawyer, 1986, 654). In this context the reader is referred to an informative study on the underlying genetics, development and evolution of *Helobdella* eyes (Kwak, *et al.* 2023).

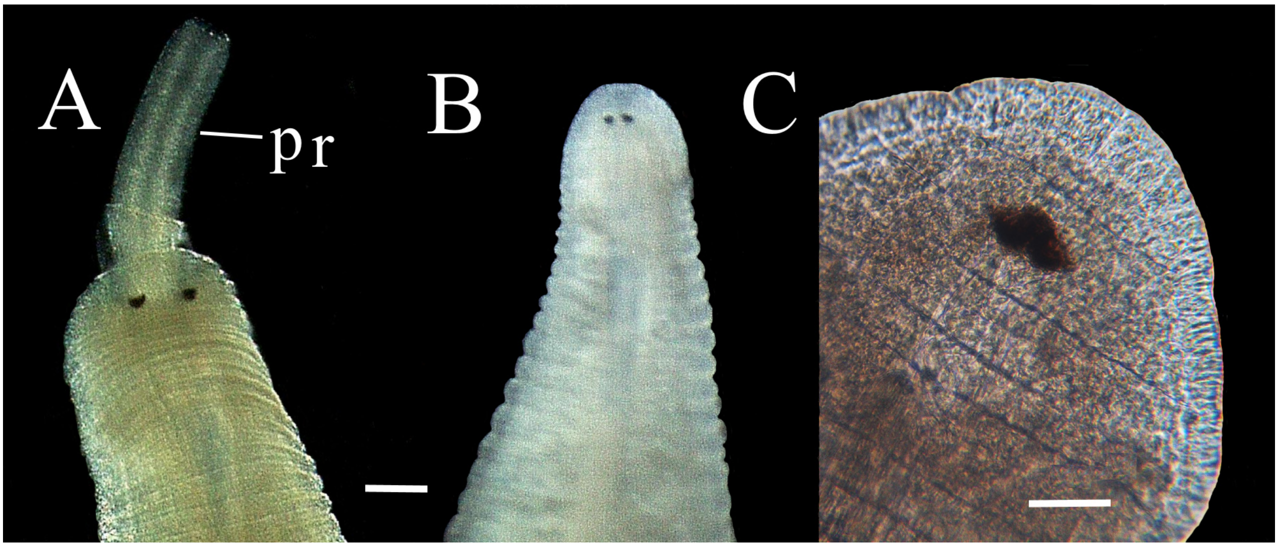

FIGURE 9. Taxonomic significance of eyes of *Helobdella* compared to *Placobdella*. A, B, *Helobdella lineata* ; C, *Placobdella multilineata*. A, Distance between eyes is maximal when the proboscis (pr) is everted. B, When the proboscis is retained internally the eyes are closer together. C, In the turtle leech *P. multilineata* the eyes coalesce physically at the mid-line and are not moveable relative to each other. Furthermore, each apparent 'eye' of *P. multilineata* on each side is composite, being comprised of a larger anterior eye and a smaller posterior eye. Scale bars: A, B, 0.3 mm; C, 0.1 mm.

The reference specimen has no visible eyes, as is the case for many of the faded specimens in this collection but in a comparable specimen (HL-15A) a single pair of well-separated eyes lies in the posterior part of segment III (i.e. on annulus III (a2+a3) (Fig. 10B). Functionally, the distance between the eyes is variable in *H. lineata* but they never adjoin at the midline. A previously unreported observation in *Helobdella* is that when the relatively large proboscis is everted the distance between the eyes adjusts spatially. In other words distance between eyes is maximal when the proboscis is everted (Fig. 9A), unlike when the proboscis is at rest, internally (Fig. 9B). In contrast the eyes of a sympatric turtle leech *P. multilineata* coalesce physically at the mid-line and are not moveable relative to each other (Fig. 9C). The immutability of the distance between eyes in the turtle leech may be attributable in part to the lack of need for spatial adjustment required by its long slender proboscis in *P. multilineata* (pers. obs.).

<u>Caudal sucker</u> The caudal sucker of the reference specimen (HL-5A) is almost circular (1.5 mm). In lateral view the posterior end of the body has a pronounced concave curve to meet the caudal sucker. This curve rides high over the centre of the sucker such that upon dorsal view only a small portion (0.24 mm) of the caudal sucker is exposed. This peculiar, somewhat humped, configuration may reflect the state of contents of the underlying large rectum.

Annulation In the reference specimen annuli of the neck region are irregularly sub-divided in keeping with extensibility of the neck (Figure 10B). On the other hand annuli of the mid-body region are more evenly expressed, but these too are occasionally subdivided. Often in some specimens (HL-5G) the subdivision is in the posterior third of the annulus rather than in the middle. In other words annular subdivision is an incompletely expressed (variable) character in *H. lineata*.

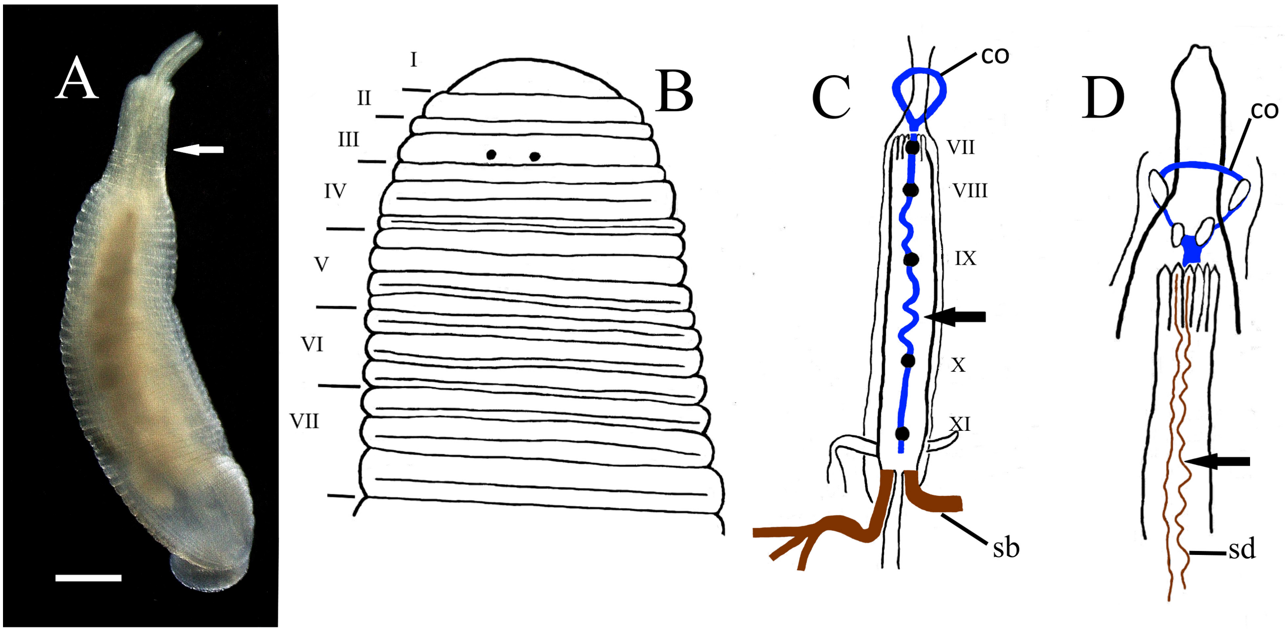

FIGURE 10. Evidence that the neck region of *H. lineata* is capable of extending and swelling (see section on feeding biology). A, proboscis everted; B–D, proboscis at rest, i.e. not everted. A (HL-9D), neck region narrows (white arrow) when proboscis is extended; B (HL-15A), annulation of the neck region at rest is more subdivided than annuli in mid-body region; C (HL-13A), ventral nerve cord (blue) between ganglia VIII–IX and IX–X is characteristically sinuous (black arrow) when individual is at rest. D (HL-13A), single salivary ductules are sinuous (black arrow) within the proboscis when individual is at rest. Abbreviations: co, circumpharyngeal commissure (brain); sd, single salivary ductule (brown); sb, salivary bundle (brown). Scale bar: 10 mm.

Incompletely expressed subdivision of annuli also occurs in the two sympatric congener species, *H. stagnalis* and *H. elongata* (Fig. 3B,C). As a variable character in all three clades annular subdivision is taxonomically unreliable and should not be used to define these or closely allied forms of *Helobdella*. In this context several 'species' of *Helobdella* and even allied 'genera' have been defined in terms of subdivided annuli, for example, *H. scutifera*, *H. longicollis* and *H. diploides* (Siddall & Borda, 2003; Ringuelet, 1978).

Internal features

The following internal description of *H. lineata* from the Albemarle region is based primarily on dissection of the reference specimen (HL-5A). This account is supplemented especially by eleven well-preserved cohorts collected together in Pasquotank County (HL-5). Six of the twelve specimens were preserved with the proboscis in typical resting position, i.e. located internally behind the brain. In the other six the proboscis is everted. All individuals had faded in the preservative so that under good lighting many internal features were clearly visible through the translucent body wall. Fortunately, the tegument of these now unpigmented individuals have become so translucent that under good lighting many internal features, especially the digestive tract, are clearly visible under the microscope. The single reference specimen (HL-5A) was dissected (Fig. 5A) only after careful external examination. Several other individuals (HL-13A, HL-8A, HL-15A and HL-6A) were also dissected and contributed to an understanding of the internal features of *H. lineata* as summarised in Fig. 11.

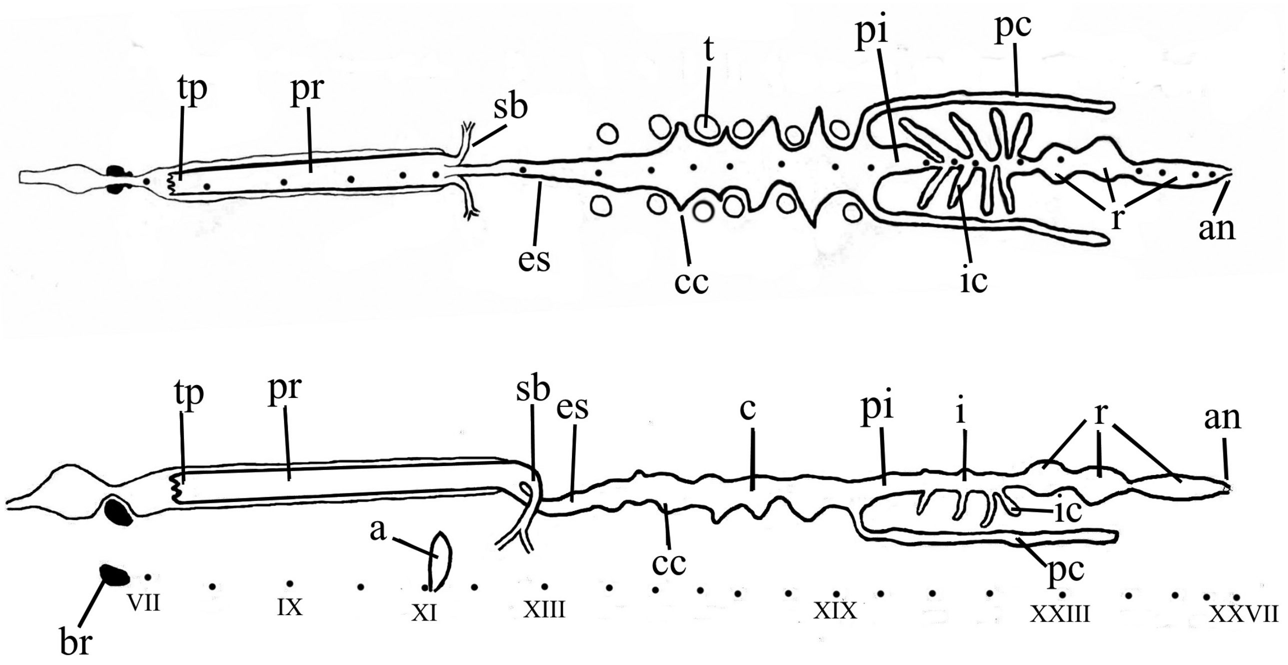

FIGURE 11. Scheme of salient features of *H. lineata*, based on dissection of the reference specimen (HL-5A, 12.8 mm). Upper, dorsal view; Lower, lateral view. Black dots refer to relative location of respective ganglia of the ventral nerve cord. Abbreviations: a, atrium; an, anus; br, brain; c, crop; cc, crop caecum (first of five pairs); es, esophagus; i, intestine; ic, intestinal caecum; pc, posterior crop caecum; pi, pre-intestine chamber; pr, proboscis; r, rectum (note: three apparent chambers); sb, salivary bundle; t, testis (third of six pairs); tp, tip of proboscis.

Foregut The following section focuses especially on specialist snail-feeding adaptations of the foregut in *H. lineata*.

Proboscis complex In its relaxed preserved state the proboscis of the reference individual (HL-5A) (Fig. 11, pr) is a straight, not particularly muscular tube. It is located at the midline from segment VII to XIII, inclusively, i.e. from just posterior to the 'brain' to beyond the male atrium. In this individual the proboscis is 3.0 mm long and about 0.6 mm in diameter through most of its length. Under good lighting a clear lumen of uniform diameter (about 85 µm) is visible through the body wall. The proboscis is housed within, but not attached to, a thin sheath which passes through the brain to join a spacious buccal cavity.

This sheath presumably allows free movement of the proboscis when it is everted (Fig. 9A), the mechanism of which remains unclear. Interestingly, half of the cohorts (HL-5G-L) collected along with the reference specimen had everted the proboscis during narcotisation. This inexplicably high rate of proboscis eversion is observed in adult individuals throughout the region. Interestingly, however, eversion of the proboscis is much less common in hatchlings of this species. For example, in cohort hatchlings of about 50 individuals (HL-5ha)(1.0 mm) only one everted its proboscis following narcotisation.

Eversion of the proboscis following narcotisation appears to be characteristic of adult *H. lineata.* In contrast, eversion of the proboscis is noticeably uncommon in the sympatric congener *H. stagnalis*.

The tip of the proboscis of *H. lineata* is a cylinder rimmed with approximately 16 stubby finger-like projections (about 80 µm in length) (Figs 9A, 12A). These and pore-like structures on the surface of the tegument, especially near the tip of the proboscis, appear to be associated with ductules of individual salivary cells (Figs 10D, 12). The tip of the proboscis is remarkably similar to that described for *H. austinensis* which reportedly has 14 finger-like projections with secretory pores (Kwak *et al*. 2021, fig. 4A). In this study the tip of the proboscis stains differentially with methylene blue.

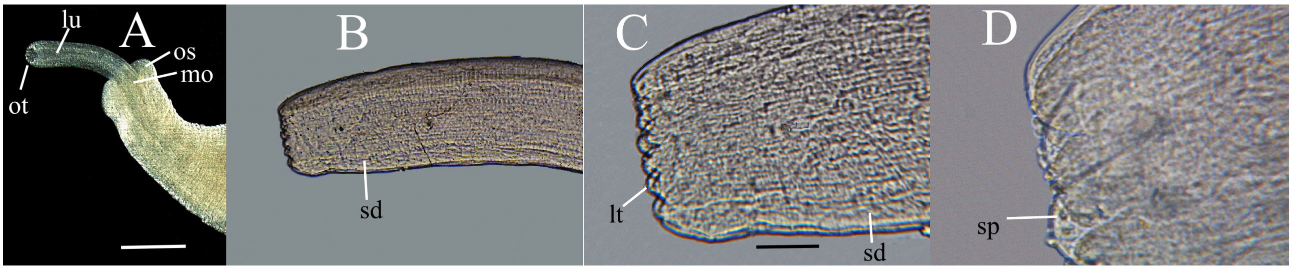

FIGURE 12. Terminal structure of the proboscis of *H. lineata* at increasing magnifications. A (HL-9L), ventral view. Everted proboscis showing unusually large mouth; tip of proboscis with circular array of lobules; the proboscis and its lumen widens near the tip. B, C, salivary ductules and close-up of lobules at the proboscis tip; D, salivary pores with secretions at tip of proboscis. Abbreviations: lu, lumen of proboscis; lt, lobule of tip of proboscis; mo, mouth; os, oral sucker; ot, opening of tip of proboscis; sd, salivary ductule; sp, salivary pores at tip of proboscis. Scale bars: A, 1.0 mm; C, 0.1 mm.

These stubby digitiform projections at the tip of the proboscis are characteristic of *H. lineata*. While serrated vestiges may appear in some *H. stagnalis* they do not appear to define this sympatric congener but this should be investigated independently.

In segment XIII the base of the proboscis divides abruptly into three parts, a medial esophagus contiguous with the lumen of the proboscis, and a bilateral pair of salivary trunks contiguous with the walls of the proboscis (Figs 11, 13A sb). On each side the salivary trunk descends, and subdivides into smaller but still stout bundles as it proceeds ventrally. In the reference individual the major salivary bundles display some degree of right and left asymmetry of no apparent taxonomic significance. These salivary bundles are comprised of individual processes emanating from the soma or body of each salivary cell. The surfaces of the larger salivary bundles are lined with distinctive diminutive cells of unclear function.

Of paramount taxonomic significance the structure of the proboscis of *H. lineata* differs fundamentally from that of the blood-feeding species *H. ghilianii*. In the former species the proboscis is short, cylindrical, non-muscular and lacks a discrete base. In the latter species the proboscis is a very long tapered, muscular structure with a bulbous base (Sawyer, 1986, 480–481).

Salivary cells The salivary cells of *H. lineata* are unexpectedly very large and often visible through the translucent body wall, especially upon staining with methylene blue. The size and locations of the individual somata of two especially clear specimens are easily compared with one another, as follows. The larger specimen (HL-5J, 12.8 mm) has approximately 79 salivary somata in total (i.e. right and left sides inclusively) (Fig. 13A, sc), whereas the smaller specimen (HL-11A, 10.4 mm) has 61 somata in total. In the former the salivary cells are distributed bilaterally over six segments from Xa3 to XVIa3, inclusively, but primarily clumped on either side at the level of, or posterior to, gXIII. The foci of the clumps on either side of segment XIII may suggest developmental origins in this somite. In the smaller individual the salivary cells are distributed over five segments XIa3 to XVa2, inclusively, but in this individual the somata are more evenly dispersed with less apparent clumping. However, in a sizeable minority of other specimens the salivary cells are close together at about gXIII and could be interpreted as various degrees of 'clumping'. In a few specimens the salivary cells may display two bilateral 'clumps'. In either case the salivary cells of *H. lineata* should be considered taxonomically 'diffuse' and not 'compact' as in the bloodfeeding allied species *H. ghilianii* (Sawyer, 1986, 485). In terms of size, the salivary somata average 102.1 µm (Range 75.1 –150.2 µm, N = 16) in the larger specimen and 108.6 µm (Range 66.2–165.4 µm, N = 16) in the smaller specimen, respectively.

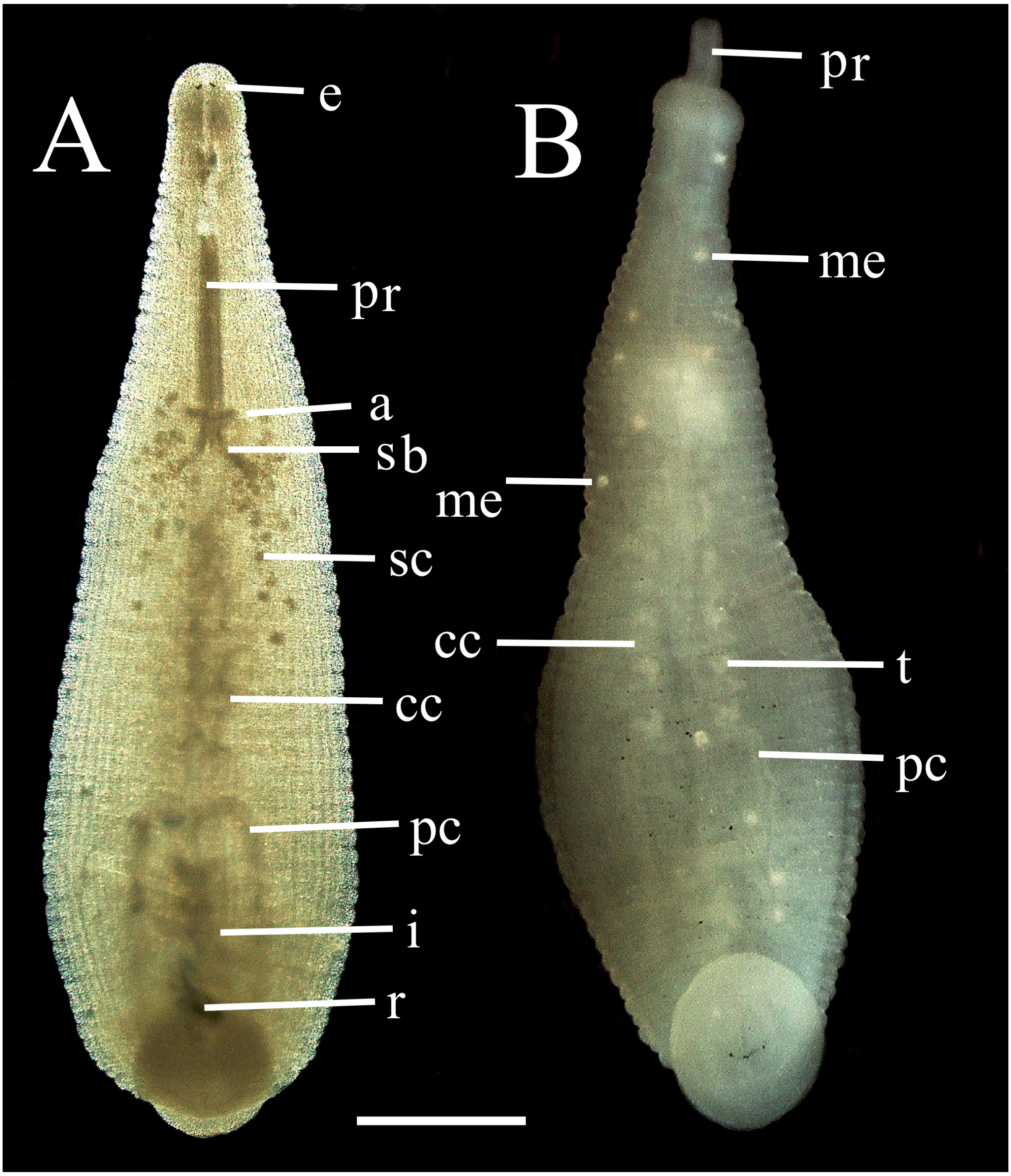

FIGURE 13. Large salivary cells. Translucent, preserved specimens of *H. lineata* showing internal morphological features of this species: A, large unicellular salivary cells (HL-11A), dorsal view; B, presumptive trematode metacercariae (HL-8B) which can be confused with salivary cells, ventral view. Abbreviations: a, atrium; cc, crop caecum; e, eye; i, intestine; pr, proboscis; me, metacercaria; pc, posterior crop caecum; r, rectum; sc, salivary cell; sb, salivary bundle; t, testis. Scale bar: 1.0 mm.

Peculiarly large salivary cells in a species of *Helobdella* were first reported by Castle (1900, 38) in what was then recognised as *H. fusca*. He observed: “The salivary glands themselves are very large in this species...”. He went on to distinguish *fusca* from the sympatric congener species *H. stagnalis* on the basis of the latter’s unremarkable salivary cells. In this context large salivary cells are herein interpreted as a specialist adaptation to feeding on snails, and arguably of considerable taxonomic significance.

The function of large salivary cells in such a diminutive leech is unproven. However, it is tempting to speculate they are linked to secretions for efficient extra-oral digestion resulting in liquidised snail tissue. In other words it is proposed they are manifestation of a snail-feeding diet incorporating extra-oral digestion.

Midgut

Esophagus The esophagus is a thin-walled tube which extends ventrally and posteriorly from the 'base' of the proboscis (Figs 5, 11, es). It has the form of a very elongated triangle in which the broad end terminates at the crop. At the posterior portion of the esophagus in the reference specimen (HL-5A) there are two barely detectable diminutive pairs of transitory lateral swellings observable only under certain conditions. Compared to the allied blood-feeding species *H. ghilianii* the esophagus of the Albemarle snail leech is remarkably short (XIII–XIV). The esophagus of *H. lineata* does not appear to be associated with bacteriomes (mycetomes), as is the case for the allied blood-feeding species *H. ghilianii* (Sawyer, 1986, 480, 517).

Apparent variations of the esophagus of *H. lineata* are associated with state of feeding. One individual (HL-9C) was preserved in the process of imbibing liquidised snail. Key photomicrographs of this process show a continuous influx of liquidised snail from the proboscis through the esophagus to the crop without demarcation between the latter two structures (Figs 14, 15).

Crop The crop of the reference specimen (HL-5A) is empty of food and is noticeably thin walled and delicate, thus making it particularly difficult to dissect. The five morphological pairs of caeca are each centred slightly anterior to its respective ganglion (XV–XIX). The caeca expand laterally with increasing width as they proceed posteriorly (Figs 5, 8 and 11, cc). In the reference specimen each caecum is basically V- or U-shaped, not forked. The tip of the fourth, and widest caecum, is noticeably pointed. The fifth and last caecum extends posteriorly as a ventro-lateral tube nearly as far as gXXIV. In the absence of food content this post-caecum has a relatively uniform diameter and constitutes a somewhat wavy tube being neither lobed, forked nor branched. This is in contrast to the blood-feeding allied species *H. ghilianii* which has seven pairs (XIII–XIX) of highly branched caeca and post-caeca (Sawyer, 1986, 480–1).

Crop caeca of snail-feeding *Helobdella* 'species' vary considerably between individuals. The differences are attributable primarily to the quantity of food (liquidised snail) pressing against thin, elastic walls of the caeca. Such crop variability is consequentially of little or no taxonomic significance in distinguishing boundaries between 'species' of *Helobdella*. For example, apparent difference in crop anatomy (smooth vs crenulated) between *H. robusta* and an allied 'species' has been proposed as species specific (Shankland *et al.* 1992, fig.1). However, this is unconvincing in that various shapes of crop caeca can be manifest even in the same individual fed at different times. For example, in this study in Fig. 8 the leading edge of the fourth crop caecum is markedly crenulated and has a prominent medial notch, whereas the fourth crop caecum of the leech in the right image of Fig. 8 displays no crenulations nor a medial notch, even though left and right images represent the same individual leech fed weeks apart.

In this study the standard number of crop caeca in *H. lineata* is consistently five pairs (Fig. 8). Another snail-feeding 'species', *H. robusta,* also has five standard pairs of crop caeca (Shankland *et al.* 1992). However, the latter species has been reported as having six pairs (Kutschera *et al*. 2013), as has *H. austinensis* (Saglam *et al.* 2023). The difference lies in the difficulty of interpreting the anatomical status of segments XIII and XIV (i.e. esophageal area) especially when food is recently ingested or partially digested (Fig. 6A, four pairs). In summary, in snail-feeding *Helobdella* the number of crop caeca per se is not a reliable taxonomic feature for defining species boundaries.

Similarly, the size and prominence of crop caeca is taxonomically unreliable in that it can be misleadingly exaggerated under some circumstances. Such over-inflated caeca are especially apparent in the posterior crop caeca which are often the last to be digested.

Pre-intestine chamber Anterior to the intestine is what appears to be a spacious, elongated chamber located between ganglia XIX and XX, respectively (Fig. 11, pi). This structure is bounded anteriorly by three pairs of conspicuous large white cells which splay downward and then up to join the intestine (HL-6A). Function of this transitory chamber between crop and intestine in this species is unknown, but appears to be more prominent than in most glossiphoniid species.

Intestine The intestine (XX–XXIII) consists of four digitiform caeca radiating away from the mid-line. In keeping with most glossiphoniids the first two pairs of intestinal caeca are directed anteriorly, the third laterally and the fourth posteriorly. Not uncommonly the intestinal caeca contain dark material presumed to be foodstuffs (liquidised snail). Interestingly, with methylene blue these intestinal caeca stain intensely.

Rectum The rectum of *H. lineata* is remarkable in structure, complexity and prominence, and also stains differentially with methylene blue. In the reference specimen (HL-5A) there are no faeces in the rectum, but overall faecal matter silhouettes that the rectum is more or less sigmoidal in shape and comprises what appears to be three consecutive chambers of roughly equal size (Fig. 11, r). The first chamber is intimate with the posterior end of the intestine, so intimate it is unclear if it may be a functional part of the intestine. The second chamber is somewhat asymmetrical and frequently filled with faeces. This second chamber narrows into a third chamber, a smooth cylinder which extends to the relatively prominent anus. In a few specimens the posterior end of the rectum is somewhat reminiscent of a swollen bladder but this requires further investigation. Functionally, the rectum of this species appears to eliminate undigested products of liquidised snail. This could account for what may be species specific complexity in the rectum of this snail-feeding leech. This contrasts with the noticeably simplified rectum of the blood-feeding allied species *H. ghilianii* (Sawyer, 1986, 480).

Feeding biology

Helobdella lineata is a specialist feeder on freshwater snails. It remains open whether this species is capable of feeding on other aquatic invertebrates especially under laboratory conditions of starvation. Nonetheless, with respect to physid snails under laboratory conditions *H. lineata* has a voracious appetite and digestion is remarkably fast, as indicated by the following observations.

The feeding habits of one isolated individual HL-26 (5.0 mm) was followed for a month in a container (d, 11 cm; h, 8 cm) with a secure lid. During this period at roughly 20 C this individual consumed an average of 1 mid-sized physid snail every 1.92 days. On another occasion this same leech was left singly with a superfluous number of snails for two weeks after which all the snails had been eaten and the gut of the leech was entirely void of content. On yet another occasion this same individual digested a complete meal in less than 4 days. Afterward this leech filled its crop in less than an hour when exposed to an additional snail.

Communal feeding A single large physid snail was placed into a container along with approximately 12 adult leeches. The snail was quickly overwhelmed by the leeches which attached their caudal suckers onto the shell and from the observer's perspective inserted their heads well into the soft parts of the snail. By morning the shell was empty without any apparent residual tissue. Nearly all of the leeches had fed on this single snail as evidenced by dark content in their guts, including juveniles carried by a brooding parent. Parenthetically, this observation confirms that *H. lineata* can conduct communal feeding as shown convincingly also for the variant *H. austinensis* (Kutschera *et al.* 2013, fig. 6; Saglam *et al*., 2023). By the next afternoon much of the gut contents was now located posteriorly, i.e., in the intestines and rectums, convincing evidence for rapid digestion in *H. lineata.*

***Helobdella* avoidance behaviour by snails** When a large physid snail is placed among *H. lineata*, the snail immediately twists its shell back and forth violently, clearly a kind of avoidance behaviour by the snail. Moreover, on several occasions it was observed that physid snails will climb entirely out of a container holding *H. lineata*. These leech avoidance behaviours by physid snails are known among other species of snail-feeding *Helobdella* and appear to display some intriguing species specificity. For example, *H. conifera* from Africa triggers avoidance behaviour by *Physa* snails but not by *Helisoma* snails. This avoidance could partially explain the lower rate of feeding by *H. conifera* on *Physa* compared to *Helisoma* under controlled conditions (Davies *et al*. 1997, 4).

Extra-oral digestion These observations on feeding by *H. lineata* highlights its inordinate capacity to digest and ingest not only the snail's haemolymph but virtually the entire solid tissue of the snail. This observation is compatible with a specialist feeding strategy in which the solid tissue of the snail is digested before it can be ingested, leaving only the indigestible shell. The remarkable thoroughness of this process suggests that powerful enzymes may be injected by the proboscis prior to ingestion. This is compatible with the finding in this study that the salivary cells of *H. lineata* are uniquely large (Fig. 13A) compared to those of its congener *H. stagnalis*, an anatomical difference first noted by Castle (1900, 38).

A distinction can be made for the first time between liquidosomatophagous feeding (sucking haemolymph) of *H. stagnalis* and allies on the one hand and extra-oral (malacophagus) digestion by *H. lineata* on the other hand. Although well known in spiders and many insects (Cohen, 1998; Walter *et al.* 2017), this appears to be the first example of extra-oral digestion in the Hirudinea.

Malacophagus feeding mechanism Photomicrographs (Figs 14 and 15) of the proboscis complex of recently fed individuals suggest that the unit of feeding in *H. lineata* is a small rounded bolus of liquidised snail (Fig. 14, b). These photomicrographs are interpreted as follows: Each bolus moves along the lumen of the proboscis into an everted sac (presumptive esophagus) at the base of the proboscis. This sac, initially outside the body, contains accumulated boluses of approximately equal size each capable of passing through the mouth. Once inside the body (Fig. 14C) the individual boluses coalesce into a larger mass (Fig. 15A, B, b) which eventually fills the esophagus and crop (Fig. 15C, es, c). This region is capable of swelling to accommodate accumulated boluses of liquidised snail (Fig. 10).

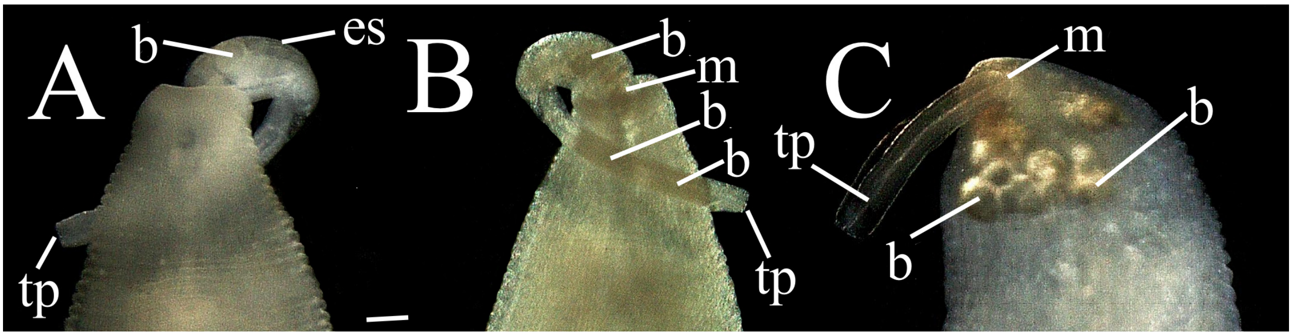

FIGURE 14. Extra-oral feeding. Evidence that *H. lineata* 'swallows' a liquidised bolus of pre-digested snail. A (HL-11D, ventral view), several boluses of liquidised snail aggregated at the proximal end of the proboscis and about to pass through the mouth. B (dorsal view of specimen A), the distal end of the proboscis contains two discrete boluses (labelled, b) near the proboscis tip, en route toward the mouth. C (HL-1B, ventral view), About 10 aggregated boluses having passed through the mouth. Note the exceptional size of the mouth in B and C. Abbreviations: b, bolus; es, esophagus; m, mouth; tp, tip of proboscis. Scale bar: 0.25 mm.

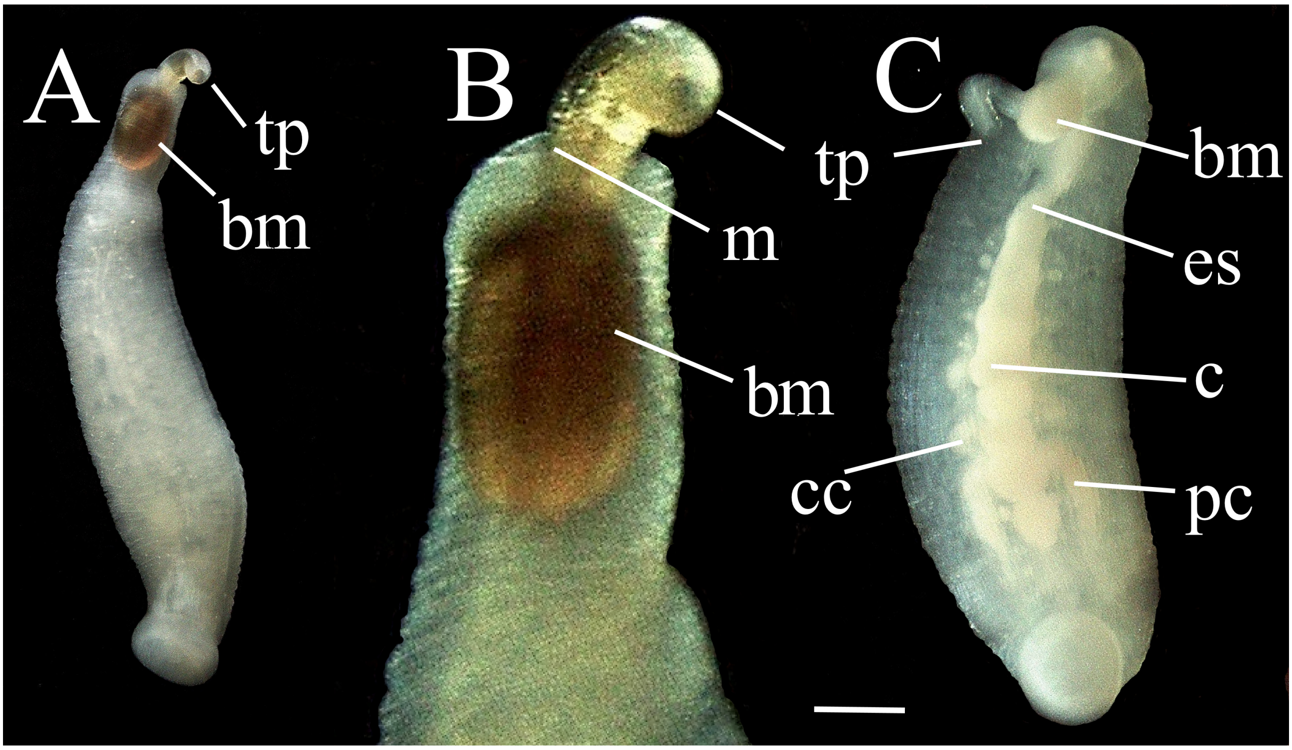

FIGURE 15. Extra-oral feeding of *H. lineata* (continued) A (HL-9A, dorsal view), boluses of liquidised snail have fused into one large bolus internal to the mouth, now incapable of passing out of the mouth. B (close-up of A). Note: compare the large size of the bolus to that of the mouth, and lumen of proboscis. C, Another individual (HL-9G, ventral view) showing continuity of liquidised snail (bm) in the post-proboscis region, much foodstuff having already filled the esophagus and crop. Note: compare swelling in the neck region shown in Fig. 10. Abbreviations: bm, bolus mass; c, crop; cc, crop caecum; es, esophagus; m, mouth; pc, posterior crop caecum; tp, tip of proboscis. Scale bar (A, C): 1.0 mm.

Reproductive Biology

Male system The male system of the reference specimen (HL-5A) of *H. lineata* can be summarised as follows (Fig. 16). The gonopore is difficult to distinguish in this specimen but is almost certainly located at the ventral mid-line at XIa3/XIIa1. From the gonopore the atrium on each side extends laterally, more or less horizontally, rather than ascending vertically as is typical of many glossiphoniids. The atrium is a relatively long and prominent structure of uniform diameter. The ejaculatory duct is a long tube, not coiled, nor apparently muscular. It is a uniformly narrow tube which extends laterally and ventrally for a short distance before bending some distance posteriorly toward the midline where it widens to form the swollen seminal vesicle. This relatively large seminal vesicle then undergoes a sharp bend anteriorly some distance before turning laterally on the same side. The two swollen seminal vesicles on each side are prominent at the ventral midline at about segments XIII and XIV. In this specimen and in a cohort individual HL-5G the seminal vesicles are somewhat whitish, or luminous under certain light.

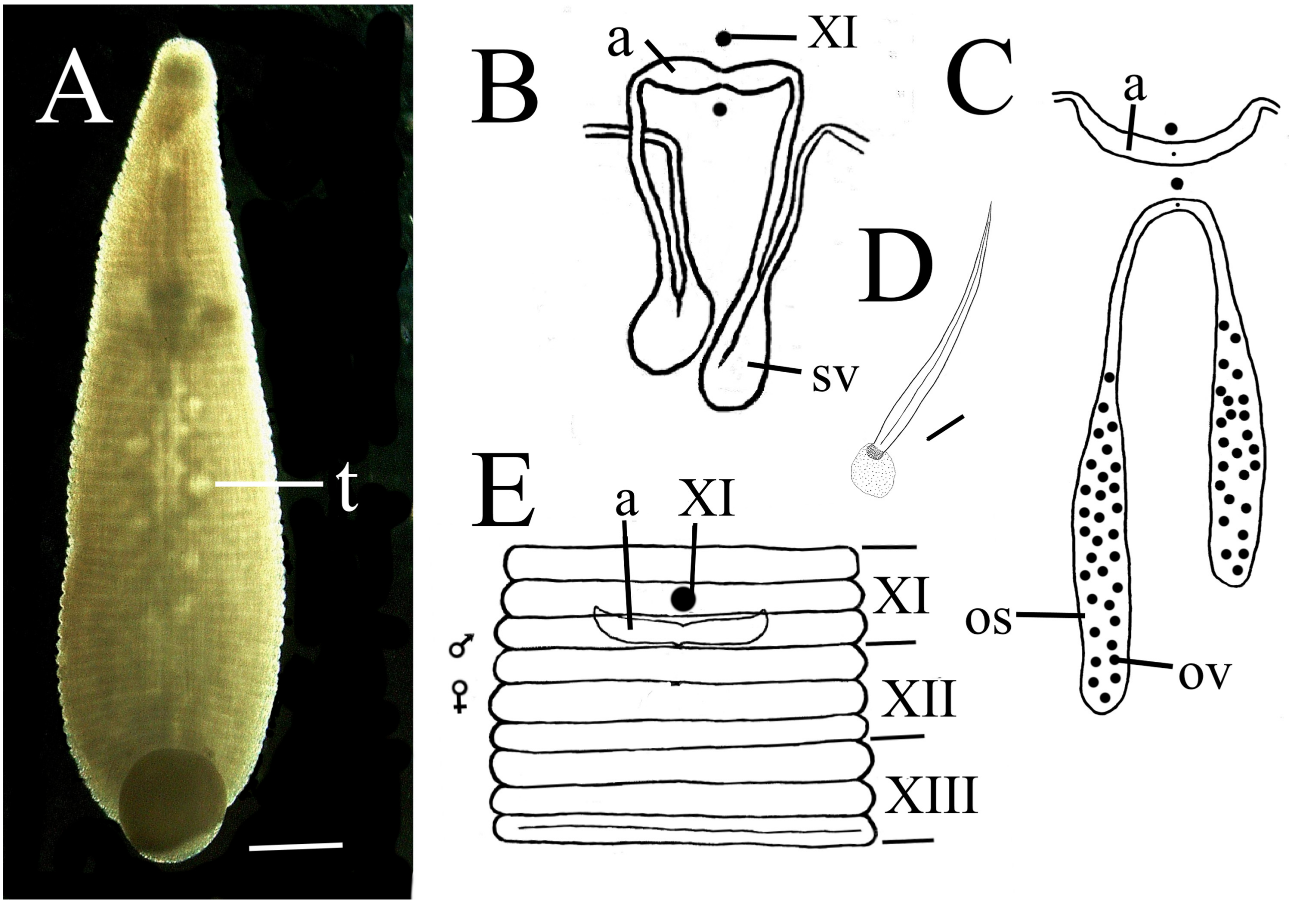

FIGURE 16. Reproductive anatomy of *H. lineata*. A, ventral view of adult (HL-5F) showing six pairs of testes; B, male structures showing atrium and seminal vesicles; C, female structures showing mature ovisac containing ova; D, spermatophore; E, external location of male gonopore at XIa3/XIIa1. Abbreviations: a, atrium; os, ovisac; ov, ovum; sv, seminal vesicle; t, testis. Scale bars: A, 1.0 mm; B–C, 100 µm.

Six pairs of testes on each side lie just posterior to ganglia XIII to XVIII, respectively (Fig. 11, t). The first two pairs are wider apart than the following four pairs. The latter lie uniformly between the first and fifth pairs of crop caeca, respectively. The sixth (most posterior) pair of testes lie posterior and lateral to ganglion XVIII, invariably in front of the posterior crop caeca. In this specimen the testes are loose, irregular spheres which stain differentially with methylene blue. In general the testes in this species are variable and difficult to distinguish. Accordingly, testes per se are not reliable taxonomic characters in this species. The vas deferens in the reference specimen is not discernible.

Of possible taxonomic significance, the male reproductive structures of *H. lineata* appear to differ from those of the sympatric congener *H. stagnalis.* For example, the atrium of the latter species faces anteriorly rather than laterally but possible species specificity cannot be pursued further here (Castle, 1900).

Fertilisation During the course of this study many *H. lineata* in the wild carried eggs or embryos on their venters. Interestingly, however, of the 404 individuals examined the act of fertilisation (i.e., impregnation by a spermatophore) was observed on only one occasion. This contrasts with frequent spermatophore impregnations in a laboratory colony of a closely related 'species' *H. robusta* (Shankland *et al.* 1992).

This single observation on *H. lineata* would be presumably an example of cross-fertilisation rather than self-fertilisation, based on size differential and on the difficult location for impregnation from its own male gonopore. This is significant in that it offers evidence that cross-fertilisation can and does occur in *H. lineata*. By way of context, individuals of snail-feeding *Helobdella* from California are capable of producing viable offspring without cross-fertilisation, as shown by David Weisblat and colleagues (Shankland *et al.* 1992; Kutschera & Wirtz, 1986; Wedeen *et al.* 1990; Iyer *et al*. 2019).

Spermatophore A small spermatophore (0.75 mm) was discovered in the tegument of an apparently gravid adult *H. lineata* (10.5 mm) in Pasquotank County (HL-8A, 13 May) some time after preservation in 5% formalin (Fig. 17). It is not known when impregnation had occurred, but it was firmly implanted into the dorsal tegument about segment XVI just anterior to the second crop caecum, about level of the right testis between crop caeca 1 and 2, and approximately half way between the mid-dorsal line and the lateral margin of the body. Most of the length of the spermatophore is bilaterally symmetrical (80 um at widest) and clearly translucent. It gradually tapers until bilaterality of the spermatophore is indistinguishable.

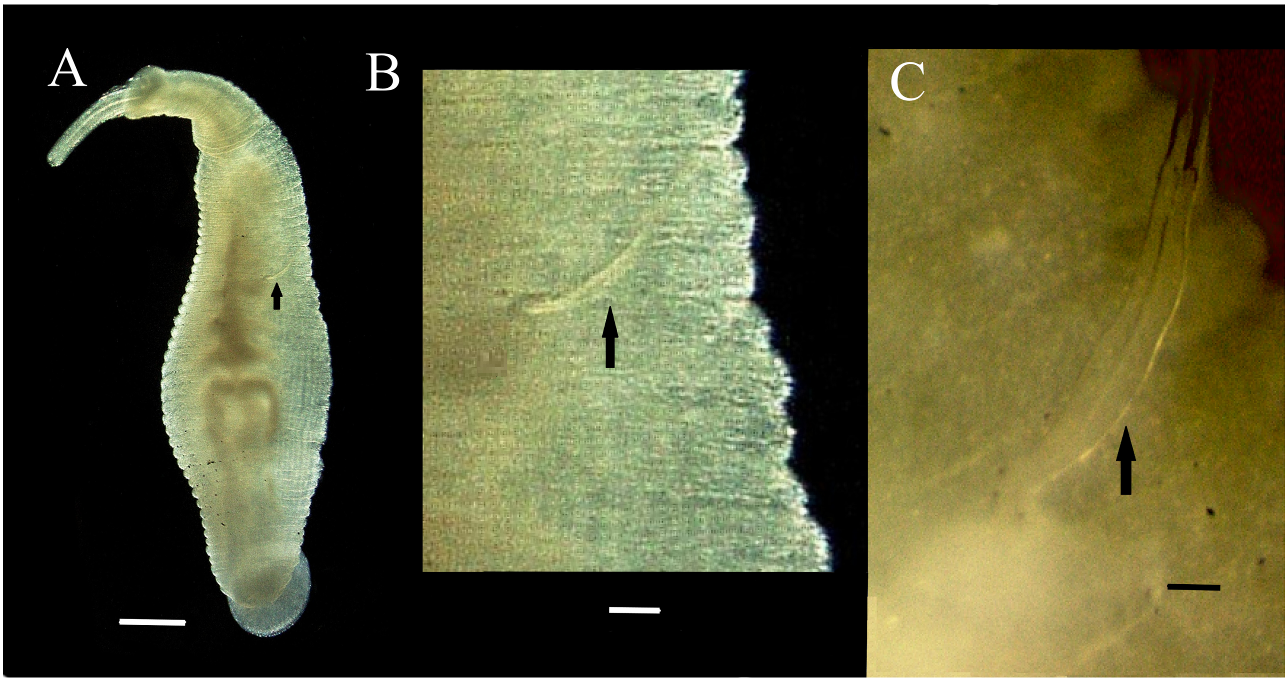

FIGURE 17. Dorsal view of an adult *H. lineata* (HL-8A) showing a spermatophore (length, 0.75 mm) (arrow) implanted in the dorsal tegument. A–C, same spermatophore at increasing magnifications. Note the tip of this spermatophore is associated with a discrete discoloured area of apparent tissue dissolution, implying enzymatic activity. Scale bars: A, 1.0 mm; B, 0.2 mm; C, 0.1 mm.

The embedded end of the spermatophore is associated with a discrete discoloured and swollen area of tissue dissolution, about the width of an annulus, implying some sort of enzymatic activity had taken place. Interestingly, this tumescent region appears to be contiguous with the crop (second crop caecum in this case). Whether there is a functional relationship between the spermatophore and crop requires further investigation. Structure of the spermatophore may have taxonomic significance (Moore, 1953) but this cannot be pursued further here.

It is noteworthy that a possible second recently fertilised individual was encountered in Tyrrell County (HL-11B). It was distinguished by a focal constriction of the body wall at the margin about segment XIX. No spermatophore was detectable in this case.

For comparison with its sympatric congener a single spermatophore was also observed implanted onto the body of *H. stagnalis* from the Albemarle (Hst-2, 23 May). This observation is significant because *H. stagnalis* (variant *octatestisaca*) is also capable of self-fertilisation (Iyer *et al.* 2019; Lai *et al.* 2009).

Female system The female gonopore of the reference specimen is difficult to distinguish but appears to be located at XIIa1/a2, one annulus posterior to the male. Internally, the non-gravid reference specimen has a pair of compact white ovisacs located at the ventral midline. In gravid specimens the ovisacs extend posterior as far as segments XVI to XVIII. Conspicuous ova (150–175 um) are sometimes visible through the translucent body wall (HL-8D, HL-10, HL-12, HL-13A) (Figs 16C and 18A). At one locality (HL-14) out of 46 adults collected, one gravid individual was unique in this study in that it bore apparent glandular tissue at the female opening manifest as a swollen mid-ventral slit behind the male atrium. It is tempting to speculate this area produces the egg sac membranes during egg deposition.

The season for egg-laying in the Albemarle region is from late spring to early summer. No breeding individuals were encountered from October/November through March, but bear in mind little collecting occurred during this winter period. The earliest gravid individual was collected on 7 April (HL-23) but gravid and brooding individuals were remarkably common every year from second half of April (HL-4, HL-6, HL-7) through May. An exhaustive analysis of fecundity was not undertaken but the following is representative. On 13 May an adult (HL-8A, 10.5 mm) bore 5 egg sacs on its venter, containing 8, 7, 7, 9 and 8 eggs, respectively, plus one dislodged egg sac containing 6 eggs. Thus, this individual laid a total of 45 eggs or an average of 7.5 eggs per egg sac in a single batch. By simple calculation a second generation could yield potentially 2025 offspring.

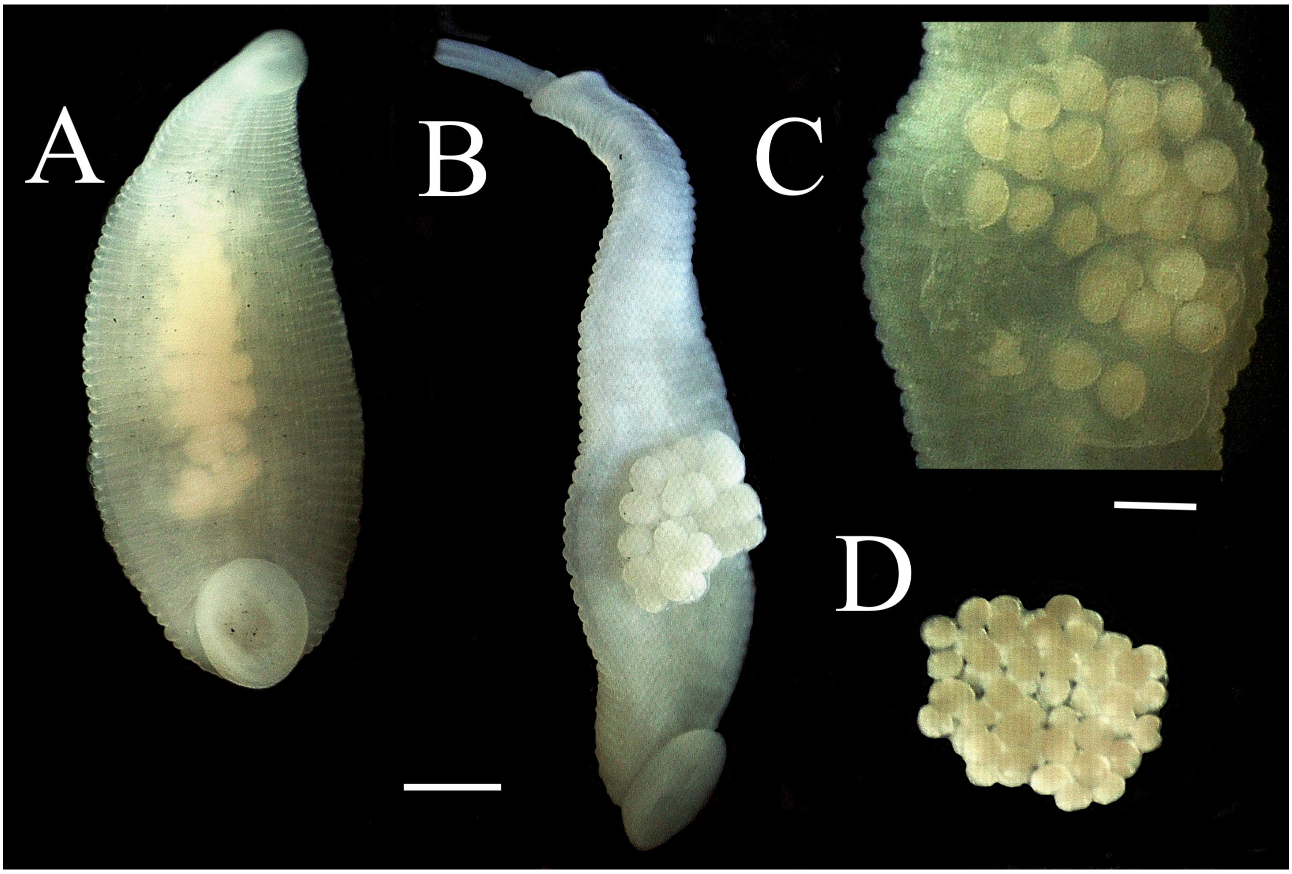

FIGURE 18. Egg laying in *H. lineata*. Yolky eggs are contained in contiguous egg sacs which are attached to venter of the parent. A (HL-8D), advanced ova in ovisacs, soon to be laid. B (HL-8G), recently deposited egg sacs on venter. C (HL-8J), close-up of early embryos emerging from egg sacs. Note characteristic body width of this brooding individual. D, several contiguous egg sacs dislodged from a brooding parent. Scale bars (approximate): (A,B) 1.0 mm, (C,D) 0.5 mm.

Based on limited information *H. lineata* may differ from its congener *H. stagnalis* in having fewer egg sacs each with more eggs. For example, one adult *H. stagnalis* from Pasquotank County (Hst-8) had 9 egg sacs with a total of 34 eggs/ embryos on its venter [left side: 5,5,5,3,1, right side: 4,5,3,3]. Interestingly, egg sacs of *H. lineata* stay attached on the venter noticeably better than those of *H. stagnalis* which are more easily dislodged. Furthermore, the membrane surrounding the egg sacs appears to be thicker and stronger than in *H. stagnalis.*

Laboratory observations, discussed elsewhere, indicate that *H. lineata* is capable of laying eggs at least three times at intervals of 41 and 38 days, respectively (20 C). Very roughly three batches at an average of 45 eggs per batch would enormously amplify the potential progeny produced by a single leech in two generations.

In a given population at a collection station the individuals are notably similar to one another. For example, in Pasquotank County (HL-8, 13 May) most of the 8 adults were about the same size (8.0–9.0 mm) and carried egg sacs at about the same stage of development (unhatched). These cohorts were larger and more advanced than 12 adults from a different nearby collection station (HL-9, 3 May) (only 'several with egg sacs').

Reproduction without cross-fertilisation? During the course of this study a single isolated individual (HL-26) (Fig. 3A) produced offspring on three consecutive occasions without intervening cross-fertilisation. This particular individual (5.0 mm) had been collected in a non-gravid state in early winter (**17 Nov**). It was captured on an aluminium leech trap (Fig. 2 B,C) and subsequently carried in isolation from the Albemarle region to the author's study in UK, where this leech was fed on laboratory-reared *Physa* at 18–22 C throughout the period of the following observations. The individual was first noticed to be gravid on 7 Jan, and on **8 Jan** cocoons were clearly attached to its venter. On 13 Jan the eggs had not hatched but the parent had recently fed, and was actively moving around. (Fig. 8, left). On 19 Jan the egg sacs looked intact, but by next day the eggs were lost, apparently unhatched. On **18 Feb** the same isolated leech laid a second batch of eggs; at 10 am, there were 2 cocoons and at 1 pm there were a total of 4 cocoons. On 27 Feb the parent was carrying live embryos. On 28 Feb the parent had moved to a new location in the container and 4–5 embryos were lying on the bottom of the container, clearly displaying peristalsis (Fig. 8, right). On 1 March no apparent embryos were on the parent, but about 6 embryos were scattered along the bottom of container, alive, moving, yolk in crop which had barely detectable caeca. During 2–16 Mar the parent was not fed, at the end of this period there was no sign of embryos. On17 Mar feeding was resumed. On 25 Mar the parent was apparently gravid. On **28 Mar** it laid a third batch of eggs, namely 4 egg sacs on its venter. On 3 Apr the eggs may have hatched but parent moved to another location. On 4 Apr all 4 egg sacs became detached from the venter; some of the embryos were pinkish but abnormal looking. Experiment discontinued (parent died).

In summary, an individual of *H. lineata* collected in the Albemarle region, and reared in isolation in UK deposited egg sacs on three occasions roughly a month apart (8 Jan, 18 Feb, 28 Mar). Overall viability of the embryos was not clearly established but active embryos were observed in at least two of the batches. While it is formally possible this particular specimen had been fertilised prior to capture in early winter when sexual activity is presumably minimal, it remains possible that none of these three batches of eggs laid by this isolated individual had been fertilised. This is relevant because reproduction without cross-fertilization ('virgin birth') is known to occur in other snail-feeding *Helobdella* from California, as mentioned above.

Ecology

Local dynamics and dispersal The peak season for growth and reproduction for *H. lineata* in the Albemarle region is late spring (16 April–31 May). A sample (N = 69) of individuals collected during the five respective weeks of the breeding period was measured and compiled (Table 1). Brief analysis of the limited data indicate that growth is very rapid during the second week of May, an increase of average length from 7.9 mm to 11.0 mm. Peak egg-laying occurs in the third week of May, the average size of individuals declines significantly from 11.0 mm to 7.1 mm (presumably due to introduction of recent offspring to the population). Growth continues in the fourth week of May, from 7.1 mm to 8.8 mm. The overall numbers captured on the aluminium traps declines from 22 in week two of May to only 4 with onset of June (attributable to increased and presumably suppressive heat of summer). Apart from the growth period of mid-May, the largest individuals are encountered coming out of the winter period (average 9.9 mm).

TABLE 1. Growth Dynamics, 2008–2019. Recorded body lengths of *Helobdella lineata* collected on aluminium traps at weekly intervals during the peak reproductive season (16 April–31 May) compiled for the 12 years of the study (see Appendix).

Dates	Mean (mm)	Range (mm)	Number
Apr 16–31	9.9	3.5–18.5	16
May 1–8	7.9	6.5–9.5	15
May 9–15	11.0	8.5–12.5	22
May 16–23	7.1	2.2–15.5	12
May 24–31	8.8	6.5–10.5	4

TABLE 2. Comparison of annual records of adults and sub-adults of two species of *Helobdella* collected in the period 15 April–31 May each year from 2008–2019 in the Gum Neck dike canal bordering Buckridge Coastal Preserve Dedicated Nature Preserve, southern Tyrrell County, North Carolina, USA [35.719874–76.107744], using aluminium leech traps (Fig. 2). Note that *H. lineata* was present every year of this longitudinal study, but *H. stagnalis* was not present for the first 10 years (2008–2017). In 2018 and 2019 not only was *H. stagnalis* present it was inexplicably more abundant than *H. lineata* both years. No *H. elongata* was found at this site in any year of the study. This longitudinal study was discontinued because of the COVID-19 pandemic.

	Helobdella lineata	*Helobdella stagnalis*
2008	**1**	**0**
2009	**present**	**0**
2010	**present**	**0**
2011	**30**	**0**
2012	**present**	**0**
2013	**7**	**0**
2014	**present**	**0**
2015	**2**	**0**
2016	**25**	**0**
2017	**20, plus ~100 juveniles**	**0**
2018	**26**	**93**
2019	**58**	**84**

The field station used in this study and adjacent waterways have been known to the author since the 1940s. For many years I have been intimately familiar with the leech fauna which live in southern Tyrrell County, dating from well before 1968 when an extensive dike system was constructed for agricultural drainage to long afterwards (post–1968 period). The main point here is that one species I never saw in these waterways back then was *H. lineata*, the subject of this paper. Based on decades of intensive collecting in this locality it is my confident opinion that *H. lineata* did not live in southern Tyrrell County until after 1968 when the dike was built. It is noteworthy that I did not find this species anywhere in Tyrrell County in my early surveys of the area (Sawyer, 1972; Sawyer and Shelley, 1976). In any case the first time I encountered *H. lineata* south of the Albemarle Sound was in November 2008.

As part of this study an annual survey of *Helobdella* species was conducted from 2008 to 2019 at the above-mentioned field station in the adjacent drainage canal [35.719874–76.107744] (Fig. 2A–C). In each of the 12 years of the study *H. lineata* was consistently captured on aluminium leech traps (Table 2). Of considerable ecological significance, its congener *H. stagnalis* was entirely absent on the leech traps for the first ten years of the current study (Table 2). Then, in May 2018 a single brooding individual of *H. stagnalis*, an easily recognisable species, was first collected at this location (Hst-1). In fact this was the first record for *H. stagnalis* in Tyrrell County, or anywhere south of the Albemarle Sound. Interestingly, gravid *H. stagnalis* reappeared the following year (Hst-3) and by then had even become established for the first time in nearby sites (Hst-4).

The conclusion from these long-term observations is that both respective species of *Helobdella* arrived in the study locality fairly recently from somewhere else. *H. lineata* appeared some time between 1968 and 2008, and *H.*

stagnalis can be dated more precisely to 2018. For the sake of future researchers the third congener in this region, namely *H. elongata*, had not been found in this specific locality by 2019. Whether these recent introductions may be attributable to changes in local agricultural practice cannot be pursued further here.

In the context of mechanisms of local dispersal in the Albemarle region, the following observation may be relevant. In late spring (19 May 2019) a mature turtle *Pseudemys floridana* was collected on land approximately 15 m from nearest water [35.719874–76.107744]. Unexpectedly, this turtle carried three individuals (HL-12) of *H. lineata* of varying sizes (11.5, 7.5, 5.75 mm). The largest individual appeared to be advanced gravid. The guts of all three had some brown content, especially in the posterior crop caeca of the largest and smallest individuals. This indicated the leeches had fed on a snail at least a day prior to getting on the turtle, the timing roughly based on independent feeding observations. There was no indication, such as red colour indicative of vertebrate blood, that any of the leeches had fed on the turtle itself. No other species of leech was on the turtle.

This relationship between snail-feeding *Helobdella* and freshwater turtles is not a new discovery. In fact, four 'variants' of snail-feeding *Helobdella* have been found on several species of turtle. The variant '*robusta'* was recently recorded on *Sternotherus odoratus* in piedmont North Carolina (Unger *et al.* 2019), not far from the current study area. In New England the nominal variant *H.* '*lineata'* was also found on the turtle *S. odoratus,* and variant *H.* '*papillata'* on the turtle *Chelydra serpentina* (Richardson *et al*. 2015). Finally, the 'variant' *H.'europaea'* has been found on different turtle species on two continents: on a land-dwelling turtle *Terrapene carolina major* in Mississippi (Richardson *et al.* 2017) and on a European species of turtle *Emys orbicularis* in Spain (Perera *et al.* 2019; Reyes-Prieto *et al.* 2013).

Global dynamics The local population dynamics of *H. lineata* and *H. stagnalis* within the microcosm of the Albemarle region addresses a much broader picture. Since the late 1970s at least one variant (*europaea*) of snail-feeding *Helobdella* has been found on all six habitable continents (Kutschera & Wirtz, 1986; Lai *et al.* 2009; Reyes-Prieto *et al.* 2013; Pederzani, 1980; Kutschera, 1985,1987, 2004; Govedich & Davies, 1998; Siddall & Budinoff, 2005; Málnás, *et al.* 2016; Morhun *et al.* 2021; Mabrouki *et al.* 2019; Ferreira *et al*. 2022; Rashni *et al.* 2023).

In other words in fewer than 50 years a snail-feeding variant of *Helobdella* has extended its range to global dimensions. In this context, historically in North America the first named species (*lineata*) of snail-feeding *Helobdella* was described in 1874 from Nebraska (Verrill, 1874). This is relatively late for finding a new species which is so commonplace today. This was followed 26 years later by a second 'species' (*fusca*) from Massachusetts (Castle, 1900). In view of the documented rapidity of global spread of closely related snail-feeding variants the question confronts us whether *lineata* and *fusca* are themselves relatively recent immigrants but this formal possibility cannot be explored further here.

Interspecific competition in the Albemarle region This study documents that three, and only three, species of *Helobdella* are indigenous to the Albemarle region, namely, *H. lineata*, *H. stagnalis* and *H. elongata* (Fig. 3). All three species feed on aquatic invertebrates but only *H. lineata* feeds preferentially on snails, whereas *H. stagnalis* and *H. elongata* feed on oligochaetes, and/or certain immature dipterans (Kutschera *et al.* 2013; Saglam *et al.* 2023; Hilsenhoff, 1963, 1964). Such apparent dietary partitioning within *Helobdella* invites further study but lies outside the scope of this paper.

Other ecological differences between the three sympatric *Helobdella* species were observed in this study. For example, the three respective species are not equally distributed in the Albemarle wetlands for reasons not well understood. For example, in the 12 years of this study *H. lineata* is by far the most abundant species of *Helobdella* in this region, and conversely *H. elongata* is barely represented at all. The total numbers of the respective species collected during this period were 404 for *H. lineata*, 198 for *H. stagnalis* and 3 for *H. elongata*. In only one of 25 collecting stations were all three species living together, namely at Hall's Creek Landing, Pasquotank County (36.219142–76.275810). Most commonly *H. lineata* was encountered by itself, without the presence of *H. stagnalis* nor *H. elongata.* Finally, it was discovered that over time the numbers of the three species living at a particular collecting station can be measurably unstable such that one species can overcome a congener over a relatively short period of time (Table 2).

Taxonomic status of snail-feeding *Helobdella* in North America Since Verrill's original description of *lineata* in 1874, a number of very similar, taxonomically confusing 'species' have been reported from the United States, Canada, Mexico and Caribbean, including *fusca* (Castle), *papillata* (Moore), *socimulcensis* (Caballero)*, punctatolineata* (Moore), *robusta* (Shankland *et al*.), *austinensis* (Kutschera *et al.*)*, europaea* (Kutschera)*,* and others (Bely & Weisblat, 2006; Siddall & Borda, 2003).

Virtually none of these ‘species’ is sufficiently known whether they meet any or all of the diagnostic criteria proposed above. Apart from *lineata* described herein, only *fusca* is known to have the requisite very large salivary cells indicative of extra-oral feeding. Indeed, in some cases it is not even known if they feed preferentially on snails.

Unfortunately, historic reliance on variable (unreliable) taxonomic criteria in some cases may undermine certainty of their identification and that of their linked molecular identification. Although this group of closely allied snail leeches is relatively common throughout much of North America surprisingly little is known of their natural history and general biology, with particular focus on their dependence on, and morphological adaptation to, feeding on snails but this cannot be pursued further here.

Insight into speciation of *Helobdella lineata*: Dietary specialisation Comparison of the feeding habits of the three indigenous species of *Helobdella* in the Albemarle region highlights the evolutionary significance of dietary specialisation. As shone in this study *H. lineata* is a specialised feeder on freshwater snails, in contrast to the apparent generalist diets of *H. stagnalis* and *H. elongata* (Hilsenhoff, 1963, 1964). Snails are undoubtedly a rich source of food but capability to feed efficiently on snails via a proboscis requires an evolutionary shift with taxonomic consequences at the species level. In the case of *H. lineata* one postulated shift is the adaptation to dissolve solid snail tissue into fluid state before the snail can be ingested and utilised fully (Figs 14 and 15). Such evolutionarily novel digestion could explain, for example, why *H. lineata* but neither *H. stagnalis* nor *H. elongata* is characterised by conspicuously large salivary cells (Fig. 13A) (Castle, 1900).

A specialised snail diet can consequentially result in other traits found in snail-feeding *Helobdella* species. For example, a diet predominantly of snails arguably results in an abundance of metabolic wastes which become assimilated into chromatophores as ‘kidneys of accumulation’, much like haemoglobin does in blood-feeding leeches (Bradbury, 1959). This could explain why *H. stagnalis* and *H. elongata*, which do not feed predominately on snails typically lack significant pigmentation (Fig. 3B,C), in contrast to *H. lineata* and its snail-feeding allies named in the preceding section.

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APPENDIX

Source material. Following are annotated locality records for three sympatric species of *Helobdella* from the Albemarle region of the Outer Banks, North Carolina, collected by the author and Dan R. Sawyer. This compilation comprises the source material for this taxonomic and morphological study of *H. lineata* (Albemarle), along with sympatric congeners *H stagnalis* and *H. elongata* from the same region (Fig. 3). Specimens prefixed (**HL**), (**Hst**) and (**HE**), respectively, are deposited in the Medical Leech Museum, Swansea, Wales, UK, and those prefixed (**USNM**) are deposited in the Smithsonian Institution, Washington, D. C.

ALBEMARLE SNAIL LEECH: *Helobdella lineata* (Albemarle)

NORTH CAROLINA

Hertford County. OLD FERRY ROAD, HARRELLSVILLE LANDING ON WICCACON RIVER [36.313998, -76.792449]: **HL-13** [12 May 2019, 1 adult, 10.5 mm (DISSECTED), brooding]

Pasquotank County. SIMONDS CREEK, ACCESS NEAR BRIDGE AT NIXONTON ROAD, NEAR ELIZABETH CITY [36.191176, -76.228516]: **HL-5** [13 May 2019, 12 adults, 12.8 mm (DISSECTED)—9.0 mm, + about 50 hatchlings]; 342 ESCLIP ROAD [36.154850, -76.170311]: **HL-8** [13 May 2019, 9 adults, 10.5mm (DISSECTED)—8.0 mm, all brooding]; HALLS CREEK LANDING, HALLS CREEK ROAD [36.219142, -76.275810]**: HL-9** [3 May 2017, 12 adults, 9.5 mm, some brooding]; **HL-10** [13 May 2019, 15 adults, gravid and brooding]
Tyrrell County, all in community of Gum Neck. STEPHEN'S RIDGE [35.721466, -76.101951]: **HL-1** [26–30 Apr 2017, 6 adults, 8.5–5.4 mm, + 10 juveniles], longitudinal striped pigment pattern; ASHBY JONES POND, INTERSECTION OF GRAPEVINE LANDING ROAD AND BUCKRIDGE ROAD [35.723628, -76.101192]: **HL-2** [9–22 May 2016, 8 adults, 1 brooding]; **HL-18** (**USNM 1191317**)[17–21 May 2011, about 5 adults deposited, 9 specimens collected]: GERALD'S POND, NEAR CHERRY RIDGE [35.707717, -76.139732]: **HL-3** [8–20 May 2016, 9 adults]; **HL-21**[8 Nov 2008, 1 adult, "first time I have ever seen this species in Gum Neck": RTS]; **HL-22** [10–23 Oct. 2013, about 16 specimens of various sizes, none brooding]; **HL-25b** [20 Mar 2012, 1 adult]; 460 GRAPEVINE LANDING ROAD, DIKE CANAL BORDERING BUCKRIDGE COASTAL PRESERVE DEDICATED NATURE PRESERVE [35.719874, -76.107744]: **HL-4** [21–26 Apr 2017,15 adults (of which 7 were photographed, 12.5–3.5 mm), 5 sub-adults, +100 juveniles]; **HL-12** [19 May 2019, 3 adult and sub-adult individuals, 11.5, 7.5 and 5.75 mm, none brooding, on Cooter turtle *Pseudemys floridana*, fifty feet from water]; **HL-14** [17–28 May 2019, 45 adults (some gravid and brooding), 8 sub-adults, 2 early juveniles]; **HL-15** [4,22 May 2018, 3 adults, 15.5 mm (DISSECTED), 11.0 mm, 7.3 mm, + 23 embryos preserved in various stages of peristalsis]; **HL-16** [8–20 May 2016, 3 sub-adults + 3 juveniles, 5.5–2.2 mm]; **HL-17** [8–20 May 2016, 1 adult, 6 sub-adults, + 12 hatchlings]; **HL-20 (USNM 1191319)** [17–22 May 2011, about 10 adults deposited (over 30 collected), among dead leaves]; **HL-23** [10–23 Oct 2013, about 7 individuals, with *Physa*, none breeding]; **HL-24** [7 Apr 2015, 1 adult]; **HL-25**a [23 Mar 2012, 1 adult]; **HL-26** [17 Nov 2015, 1 adult, 5.0 mm]; STREAM AT COOPER CORNER, BELOW CULVERT, 5632 GUM NECK LANDING ROAD [35.712749, -76.114145]**: HL-11** [22–27 May 2019, 4 adults, 10.4–6.0 mm]; DRAINAGE DITCH NEAR FREE WILL BAPTIST CHURCH, BELOW CULVERT UNDER GUM NECK LANDING ROAD NEAR INTERSECTION WITH GRAPEVINE LANDING ROAD [35.717601, -76.116115]: **HL-19** (**USNM 1191318)** [17–22 May 2011, 5 adults]; **HL-25c** [20, 21 Mar 2012, 1 adult, 2 sub-adults, + 'lots of snails']

Wayne County. WILLIAMS MILL POND, AT NC HIGHWAY 55 AT NORTHEAST CAPE FEAR RIVER [35.187120, -77.984474] Williams Mill Pond is noteworthy in that it has a long shoreline with oxygen-rich wave action: **HL-6** [28 April 2018, 4 adults, 18.5 mm (DISSECTED)—7.5 mm, + 80 hatchlings, largest three individuals in study]; **HL-7** [18 Apr 2017, 1 adult, 7.0 mm, brooding, longitudinal striped pigment pattern]

SYMPATRIC CONGENERS

Helobdella stagnalis

Tyrrell County. GUM NECK, DIKE CANAL AT 460 GRAPEVINE LANDING ROAD, BORDERING BUCKRIDGE COASTAL PRESERVE DEDICATED NATURE PRESERVE [35.719874, -76.107744]: **HST-1** [4 May 2018, 1 brooding adult, 11.0 mm, + 12 juveniles, first record from Tyrrell County]; **Hst-2** [4–13 May 2018, 18 adults, some brooding, 19 juveniles, 43 hatchlings]; **Hst-3** [17–28 May 2019, 18 adults (several gravid), 11 juveniles, 40 hatchlings]; GUM NECK, STREAM AT COOPER CORNER, BELOW CULVERT, 5632 GUM NECK LANDING ROAD [35.712749, -76.114145]: **Hst-4** [24 May 2019, 2 adults]

Hertford County. NEAR HARRELLSVILLE, ON OLD FERRY ROAD (STATE ROAD 1433), BOAT LANDING ON WICCACON RIVER, OFF CHOWAN RIVER [36˚ 18’ 49.6” N, 76˚ 47’ 32.4”W]: **Hst-5** [12 May 2019, 3 adults, 1 juvenile]

Pasquotank County. HALLS CREEK LANDING, HALLS CREEK ROAD [36.219142, -76.275810]: **Hst-6** [3 May 2017, 1 brooding adult, with 22 hatchlings]; SIMONDS CREEK, ACCESS NEAR BRIDGE AT NIXONTON ROAD, NEAR ELIZABETH CITY [36.191176, -76.228516]; **Hst-7** [13 May 2019, 1 adult]; 342 ESCLIP ROAD [36.154850, -76.170311]: **Hst-8** [13 May 2019, 1 adult brooding 9 cocoons containing a total of 34 eggs]

Helobdella elongata

Pasquotank County. HALLS CREEK LANDING, HALLS CREEK ROAD [36.219142, -76.275810]: **HE-1** [3 May 2017, 1 brooding adult, 12.5 mm, 17 eggs retained]; **HE-2** [1 May 2018, 2 adults, 12.0 mm, 12.6 mm]