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Temnosewellia semperi (Platyhelminthes: Temnocephalida) from *Maydelliathelphusa lugubris* (Arthropoda: Gercarcinucidae) of the state of Meghalaya, North East India

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ABSTRACT: Temnocephalids are rhabdocoel flatworms that are found either commensal or parasitic on a wide variety of hosts. In India, the occurrence of temnocephalids was recorded and studied from preserved specimens. Regarding the morphology of the specimens, vague descriptions were given. Since then, no following studies were found. This study provides a new insight regarding the specimens found on a freshwater crab, *Maydelliathelphusa lugubris* (Wood-Mason, 1871). The specimens are identified as *Temnosewellia semperi* (Weber, 1890) based on the presence of brown pigment, five tentacles, posterior adhesive disc, five syncytial plates: tentacular, postentacular, body, peduncular and adhesive plates, the excretory pore lying on the dorsal side of the body plate and the absence of papillae on the dorsal surface or tentacular surface. The most important diagnostic feature is the structure of the cirrus which is the main taxonomic character to identify the species. *Temnosewellia semperi* has a nearly straight cirrus with the distal part slightly dilated as observed in this species.
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KEY WORDS: Crustacea, North East India, Platyhelminthes, Temnocephalida, taxonomy, ectosymbiosis.

Temnosewellia semperi (Platyhelminthes: Temnocephalida) с *Maydelliathelphusa lugubris* (Arthropoda: Gercarcinucidae) в штате Мегалая, Северо-Восточная Индия

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РЕЗЮМЕ: Темноцефалиды — рабдоцеллидные плоские черви, которые могут быть комменсаллами, либо паразитируют на самых разных хозяевах. Темноцефалиды ранее были зарегистрированы в Индии и изучены по сохранившимся образцам. По этим экземплярам были даны расплывчатые описания их морфологии. С тех пор не было опубликовано никаких работ по темноцефалидам Индии. Настоящее исследование позволяет по-новому взглянуть на образцы, обнаруженные на пресноводном крабе *Maydelliathelphusa lugubris* (Wood-Mason, 1871). Экземпляры идентифицированы как *Temnosewellia semperi* (Weber, 1890) на основании наличия коричневого пигмента, пяти щупалец, заднего прикрепительного диска, пяти синцитиальных пластин (щупальцевых, посттентакулярных, пластин тела, ножек и адгезивных), выделительной поры, лежащей на дорсальной стороне тела; а так же на основании отсутствия сосочков на дорсальной поверхности или щупальцевой поверхности. Наиболее важным диагностическим признаком служит структура цирруса, который является основным таксономическим признаком для идентификации вида. *Temnosewellia semperi* имеет почти прямой циррус с немного расширенной дистальной частью, как это характерно для этого вида.

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КЛЮЧЕВЫЕ СЛОВА: ракообразные, Северо-Восточная Индия, Platyhelminthes, Temnocephalida, таксономия, эктосимбиоз.

Introduction

Temnocephalids are rhabdocoel turbellarians that are ectosymbionts on a variety of freshwater host species ranging from molluscs, crustaceans and turtles (Seixas *et al*., 2010; Cannon, 1991; Brusa, Damborenea, 2000). They are characterized by a multisynctial epidermis, divided into a series of plates, presence of a posterior adhesive organ and a tendency to lose locomotor cilia (Joffe, Cannon, 1998). Besides, they also have eyes with red pigments and excretory pores enclosed in the excretory plates (Damborenea, Cannon, 2001). The main taxonomic character to differentiate the *Temnocephala* species is the cirrus. So far, these turbellarians are mainly known and recorded from South America and Australia. In 1924, Haswell reported six tentacled and five tentacled with brown pigment temnocephalids occurring on crabs, crayfish and shrimps. These temnocephalids with brown pigment were later kept under the genera *Temnohaswellia* for six tentacled (Pereira, Cuocolo, 1941) and *Temnosewellia* for five tentacled (Damborenea, Cannon, 2001). The genus *Temnosewellia* includes about 50 species (Cannon, 1991; Cannon, Sewell, 2001; Damborenea, Cannon, 2001; Sewell *et al*., 2006) characterized by five anterior tentacles, posterior adhesive disc, without papillae on the dorsal surface or tentacular surface, with dark pigment and with five syncytial plates: tentacular, postentacular, body, peduncular and adhesive plates; the excretory pore lies on the dorsal side of the body plate. *Temnosewellia* is distributed throughout the Australian region. Few species were also recorded in the oriental region (Gelder, 1999). In India, the occurrence of *Temnocephala semperi* was reported on tortoises from Narmada river (Madhya Pradesh) and freshwater crabs *Paratelphusa* (*Barytelphusa*) *lugubris* from Myntang river, Jaintia Hills (Assam, presently Meghalaya) although the exact locations in both cases are unknown as the study was made on preserved specimens available in the Zoological Survey of India, Kolkata (Chauhan,

Ramakrishna, 1953). The detailed descriptions of these temnosewellids are not available in the paper since they were documented from preserved specimens. The colour of these specimens was not discernible on account of long preservation in spirit and the only morphological account that was given was the shape of the adhesive disc which was oval. Thus in the present study, for the first time, we describe the live specimens of temnosewellids from Meghalaya, North East India, associated with the crustacean, *Maydelliathelphusa lugubris* (Wood-Mason, 1871) (Fig. 2A–B).

Materials and Methods

Four specimens of *Maydelliathelphusa lugubris* (Wood-Mason, 1871) (crab, earlier known as *Paratelphusa* (*Barytelphusa*) *lugubris*) were manually collected from the freshwater stream called Umjajew stream, Mawklot, Upper Shillong, Meghalaya, India (25°33′18″N 91°49′40″E). Specimens of temnosewellids were removed from the lateral side of the body as well as from the chelipeds of host crabs under a stereomicroscope, rinsed in saline solution, and preserved in cold alcohol-formalin-acetic acid (AFA) and 70% ethanol for morphological identification by light microscopy. Morphology of the male and female reproductive system was studied by light microscopy in specimens stained in Borax carmine. Two specimens of *Maydelliathelphusa lugubris* were sent alive to Zoological Survey of India, Regional Centre, Shillong for identification using taxonomic keys. The temnosewellids were identified by focusing on the morphology of the reproductive complex and the shape of the dorsolateral excretory syncytial plates (DLSPs). The terminology used to describe the cirrus of the temnosewellids was compatible with the previous descriptions (Weber, 1890; Sewell *et al*., 2006; Damborenea, Brusa, 2009; Seixas *et al*., 2011; Garcés *et al*., 2013; Ponce de León *et al*., 2015). Measurements were made in micrometres (μm) unless otherwise indicated using microview software; ranges were determined followed by the arithmetic mean, the standard deviation and the number of specimens measured for a given character (mean, standard deviation). Photomicrographs of the temnosewellids were taken with Olympus CX41RF Trinocular Phase Contrast microscope. Type specimens were deposited in the Museum of the Department of Zoology, Shillong College (DZSC), and National Repository housed in Regional Centre, Zoological Survey of India (IV/NERC/ZSI-330), Shillong.

Taxonomy

Phylum: Platyhelminthes Minot, 1876
Order: Rhabdocoela Ehrenberg, 1831
Family: Temnocephalidae Monticelli, 1899
Genus: *Temnosewellia* Damborenea et Cannon, 2001
Temnosewellia semperi (Weber, 1890)
Figs 1–5.

TYPE HOST AND TYPE LOCALITY: *Telphusa* sp. from Sumatra, Java and various parts of Celebes (Weber, 1890).

OTHER HOSTS AND LOCALITIES: Tortoises (did not mention the genus) from Narmada river (Madhya Pradesh) and *Paratelphusa* (*Barytelphusa*) *lugubris* from Myntang River, Jaintia Hills (Assam, presently Meghalaya) (Chauhan, Ramakrishna, 1953) (Fig. 1A).

HOST IN PRESENT WORK: *Maydelliathelphusa lugubris* (Wood-Mason, 1871).

Site of infection. Lateral sides of the body and chelipeds.

Prevalence. 50% of the eleven hosts were infected.

NEW LOCALITY (PRESENT WORK): Umjajew stream, Mawklot, Upper Shillong (25° 33′18″N 91°49′40″E), East Khasi Hills District, Meghalaya, India. The stream located near Nongumlong-Mawklot road is a freshwater stream, with a width of around 1.8 m and a length of 1.5 km where it merges with the Umiam river (Fig. 1B). The stream is perennial although the volume of water increases from July till August due to regular rainfall. Collection of freshwater crabs, *Maydelliathelphusa lugubris* infested with temnosewellids were

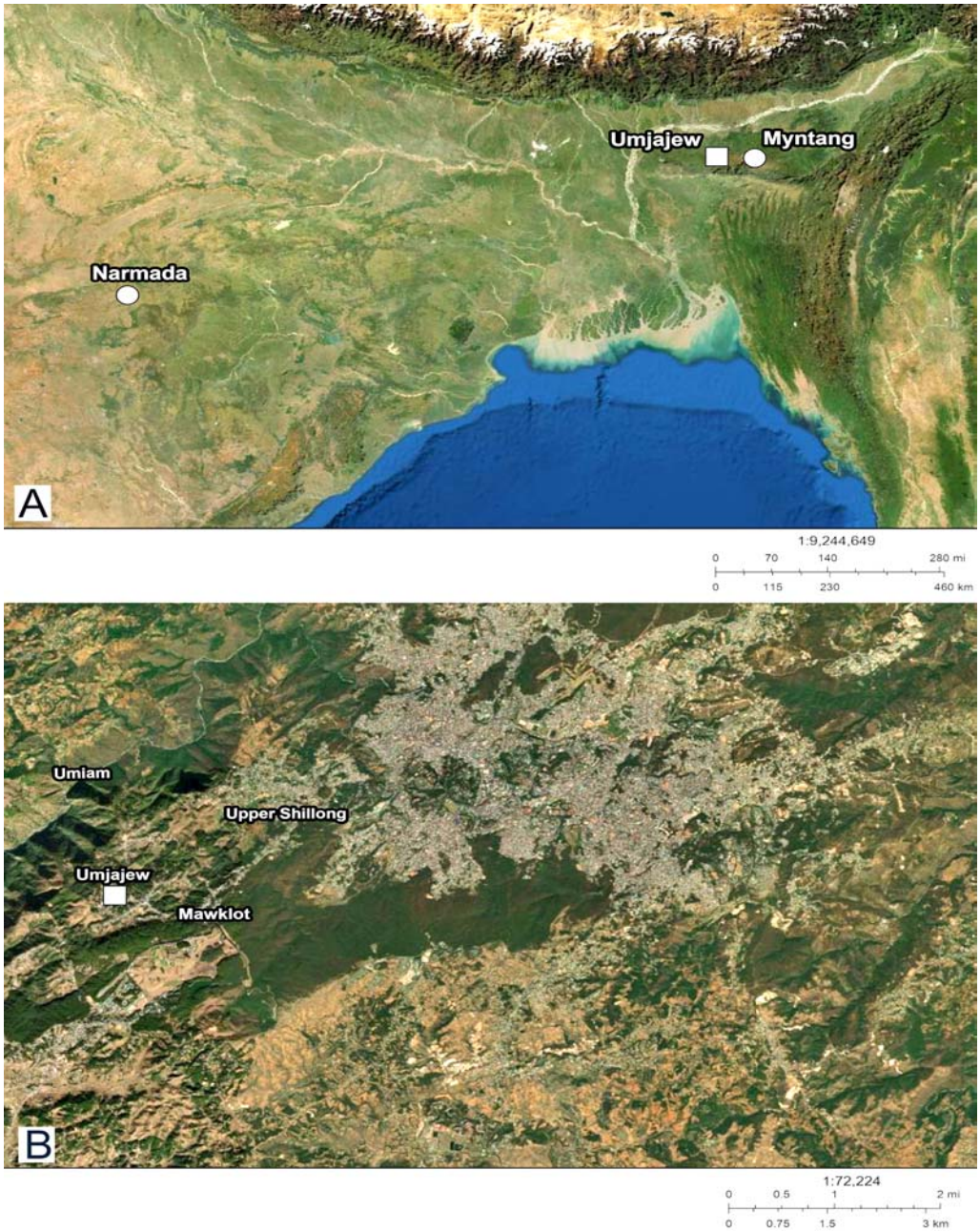

Fig. 1. Sampling locations. A — map showing the distribution of *Temnosewellia semperi* (Weber, 1890) in the Indian sub-continent. Previously known localities marked with a circle and the new locality marked with a square symbol; B — map showing the exact location of the new locality (present work), Umjajew stream marked with a square.

Рис. 1. Места находок. A — карта, показывающая распространение *Temnosewellia semperi* (Weber, 1890) на Индийском субконтиненте. Ранние находки обозначены кружками; новые находки отмечены квадратиками; B — карта, показывающая точные координаты новых находок (настоящая работа), ручей Umjajew отмечен квадратом.

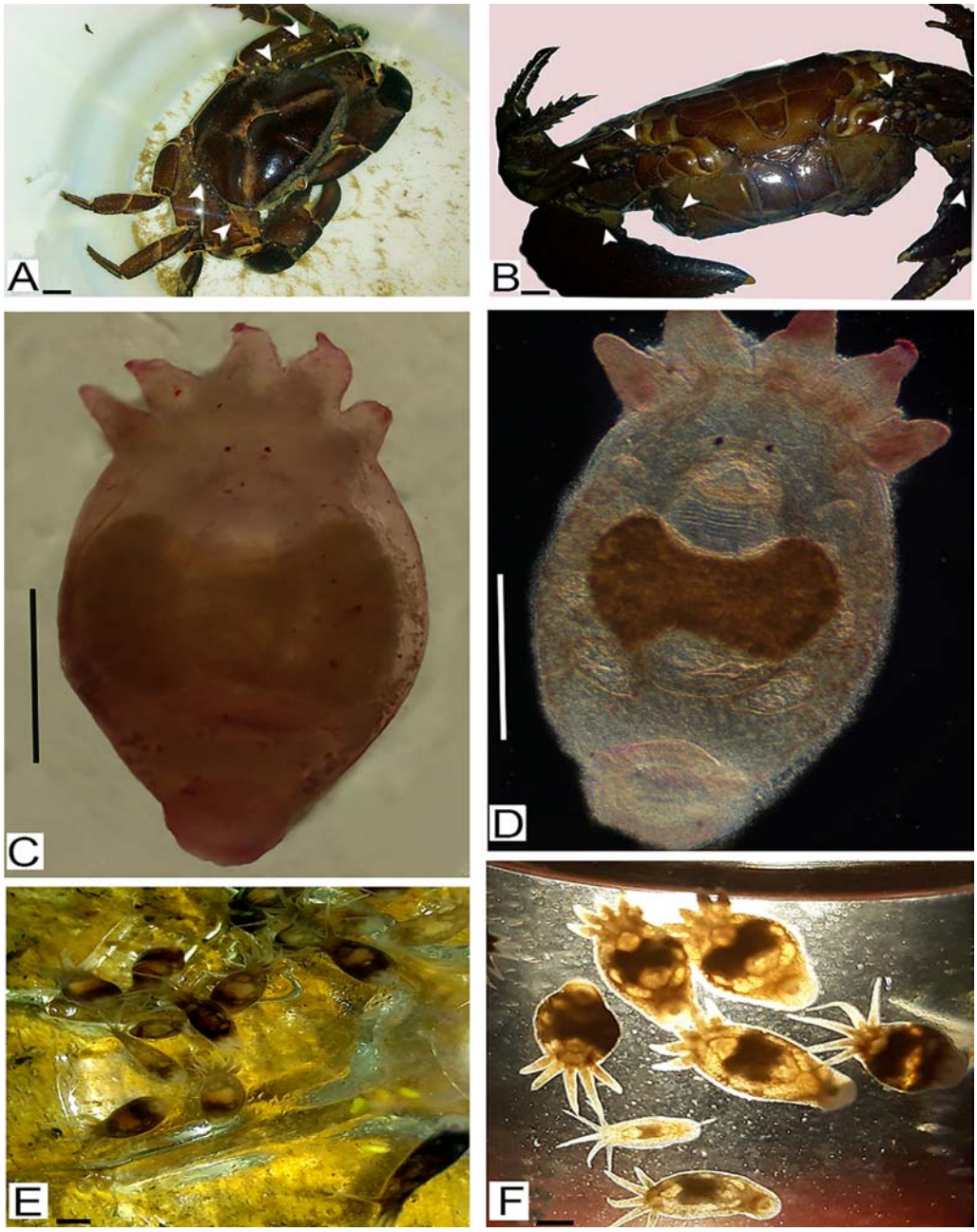

Fig. 2. *Temnosewellia semperi* and their host *Maydelliathelphusa lugubris*. A–B — specimen of *M. lugubris* (carapace diameter: 40–60 mm) infested with *T. semperi* (white arrows); C — paratype of *T. semperi* (ventral view); D — holotype stained in Borax carmine; E–F — live specimens of *T. semperi*. Scale bars: A, B — 2.5 mm; C, D — 1 mm; E, F — 5 mm.

Рис. 2. *Temnosewellia semperi* и его хозяин *Maydelliathelphusa lugubris*. A, B — экземпляр *M. lugubris* (диаметр карапакса 40–60 мм), зараженный *T. semperi* (белая стрелка); C — паратип *T. semperi* (вентрально); D — голотип, окрашенный борным кармином; E, F — фото живых *T. semperi*. Масштаб: A, B — 2,5 мм; C, D — 1 мм; E, F — 5 мм.

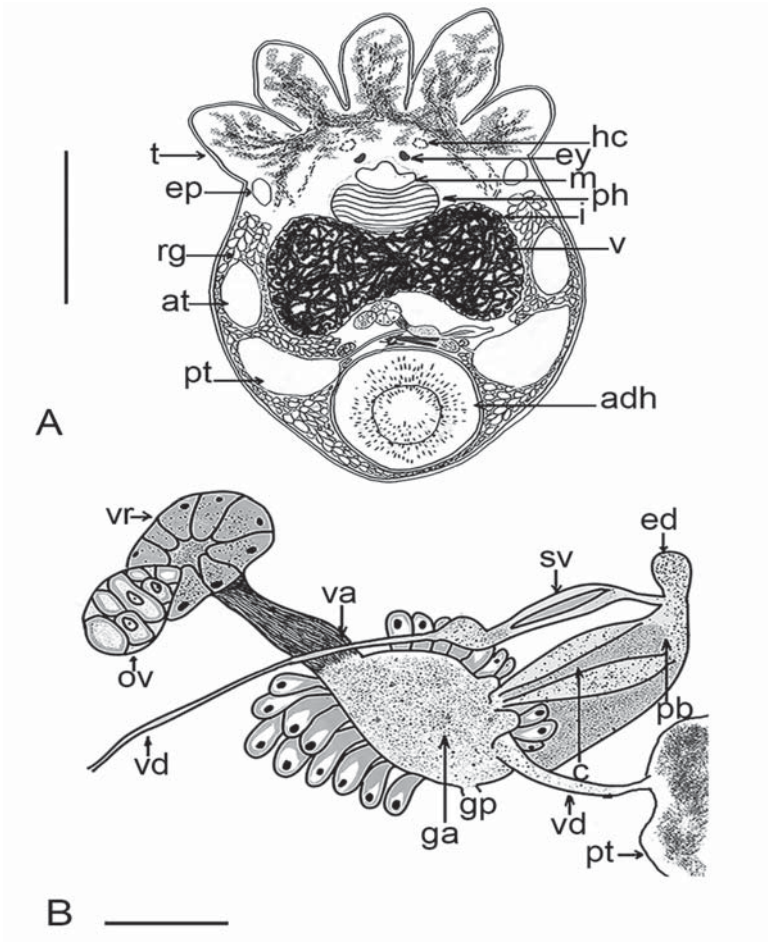

Fig. 3. Schemes of organization of *Temnosewellia semperi* (Weber, 1890). A — adult specimen diagram showing adhesive disc, posterior testes, anterior testes, rhabditogenic glands, excretory pores, tentacles, Haswellrs cells, eyes, mouth, pharynx, intestinal sac, and vitellarium; B — a diagram on the reproductive system, showing female reproductive complex: ovary, vesicula resorbens, vagina, and male reproductive complex: posterior testes, vasa deferentia, seminal vesicle, ejaculatory duct, prostatic bulb, cirrus, genital atrium and genital pore.

Abbreviations: adh — adhesive disc; at — anterior testes; c — cirrus; ed — ejaculatory duct; ep — excretory pores; ey — eye; ga — genital atrium; gp — genital pore; hc — Haswellrs cells; i — intestinal sac; m — mouth; ov — ovary; pb — prostatic bulb; ph — pharynx; pt — posterior testes; rg — rhabditogenic glands; sv — seminal vesicle; t — tentacles; v — vitellarium; va — vagina; vd — vasa deferentia; vr — vesicula resorbens. Scale bars: A — 1 mm; B — 150 μm.

Рис. 3. Схемы строения *Temnosewellia semperi* (Weber, 1890). A — схема взрослого животного, показывающая прикрепительный диск, задние и передние семенники, рабдитогенные железы, экскреторные поры, щупальца, Haswellrs cells, глаза, рот, глотку, кишечный мешок и желточник; B — схема репродуктивной системы показывающая женский репродуктивный комплекс: яичник, vesicula resorbens, вагина, и мужской репродуктивный комплекс: задние семенники, семявыносящий канал, семенной пузырек, семяизвергательный канал, бульба простаты, циррус, половой атриум и половая пора.

Обозначения: adh — прикрепительный диск; at — передний семенник; c — цирус; ed — эякуляторный канал; ep — экскреторная пора; ey — глаза; ga — половой атриум; gp — половое отверстие; hc — Haswellrs cells; i — кишечник; m — рот; ov — яичник; pb — бульба простаты; ph — глотка; pt — задний семенник; rg — рабдитогенные железы; sv — семенной пузырек; t — щупальца; v — желточник; va — вагина; vd — семявыносящий проток; vr — vesicula resorbens. Масштаб: A — 1 mm; B — 150 μm.

made on the 28th September 2019 by N.G Kharir.

TYPE MATERIAL — Holotype: wholemount specimen (DZSC-20). Paratypes: 5 specimens (IV/NERC/ZSI-330).

EXAMINED MATERIAL — Same data as type material, 5 whole mount specimens stained in borax carmine (DZSC-20); unhatched eggs, and 20 specimens in 70% ethanol (DZSC-21); 10 specimens in AFA(DZSC-22).

Description

EXTERNAL CHARACTERISTICS: Body (without tentacles) 2.0–3.9 mm (2.9±0.42) long by 1.0–2.0 mm (1.47±0.19) wide; adhesive disc ventral, subterminal, pedunculated with a disc diameter of 564–690 µm (588± 69) at rim and 240–300 µm at disc penducle (280±20) (Figs 2 C–D, 3A, 4E); eyes with black pigmentation (Figs. 2C–D, 4A).

EXCRETORY SYSTEM: Two elliptical excretory syncytial plates lying dorsolateral to the mouth, outside the post tentacular syncytial plates are present (Figs 2C–D, 4C–D). The right excretory plate is 165–218 µm (183±25) long and 90–130 µm (115±30) wide whereas the left excretory plate is 106–130 µm (120±10) long and 70–100 µm (85±13) wide.

ALIMENTARY SYSTEM: Mouth surrounded by a large muscular sphincter 100–140 µm (126±29) long by 220–257 µm (230±27) wide; pharynx 200–230 µm (226±56) long by 420–465 µm (447±55) wide (Fig. 4A); intestine saccular, without septations (Figs 2C–D, 3A).

GLANDS: Bunches of rhabditogenic glands are present in the lateral sides of the body encompassing from the pharynx to the middle level of the adhesive disc. Haswell's cells in front of the eyespots and the brain (Figs 2C–D, 4B).

FEMALE REPRODUCTIVE SYSTEM: Gonopore mid-ventral, in the posterior third of the body (Fig. 3A); surrounded by a muscular sphincter. Ovary unpaired, small and lying adjacent to the vesicula resorbens; vesicula resorbens is elliptical and indenting towards the intestinal sac; vagina large and muscular, connects to the spacious genital atrium (Figs 3B, 5A). Eggs yellow, without peduncle, deposited over the host carapace and on the chelipeds, 500–550 long (527±21), 220–310 µm wide (258± 38), slightly reniform, polar filament extremely short (Fig. 5D–E).

MALE REPRODUCTIVE SYSTEM: Two pairs of testes are present; anterior pair round and lateral to intestine sac; posterior pair always lateral and posterior to intestine, oblique, elliptical; bigger than anterior testes (Figs 2D, 3A). Right anterior testis 123–236 µm (196±51) long, 102–139 µm (122±15) wide; right posterior testis 321–399 µm (348±37) long, 156–240 µm (193±36) wide; left anterior testis 162–226 µm (195±26) long, 109–126 µm (118±7) wide; left posterior testis 312–379 µm (340±31) long, 176–227 µm (195±23) wide. Vasa deferentia wide swollen, uniting separately to a large, pyriform, thick-walled, seminal vesicle. Ejaculatory sac present, with narrowed neck. Prostate bulb not well defined, as a continuation of the cirrus base (Figs. 3B, 5A). Cirrus almost straight (Fig. 5A–B), 182 µm long; 128 µm shaft length; 48 µm wide at proximal end; 15 µm wide at distal end. Introvert 54 µm long, swollen and provided with numerous spines. The reversible spined introvert has an evident unspined distal region (Fig. 5C). The ratio between total length of cirrus and maximum width of shaft's proximal end 3.79; ratio between total length of cirrus and total length of introvert 3.37. Introvert spines very small, in approximately 30–40 parallel rows arranged slightly diagonal to the long axis of the introvert (Fig. 5C).

Discussion

The presence of temnocephalids in the Indian region was recorded only in 1953 (Chauhan, Ramakrishna, 1953). Although the paper reported the occurrence of *Temnocephala semperi* from Narmada river and Jaintia Hills but it provided only a vague description. The original description of the specimen is lacking except for the nature of sucker and length of the body which have no systematic values. As reported in the paper, pigmentation on the specimens could not be determined as they were preserved for a

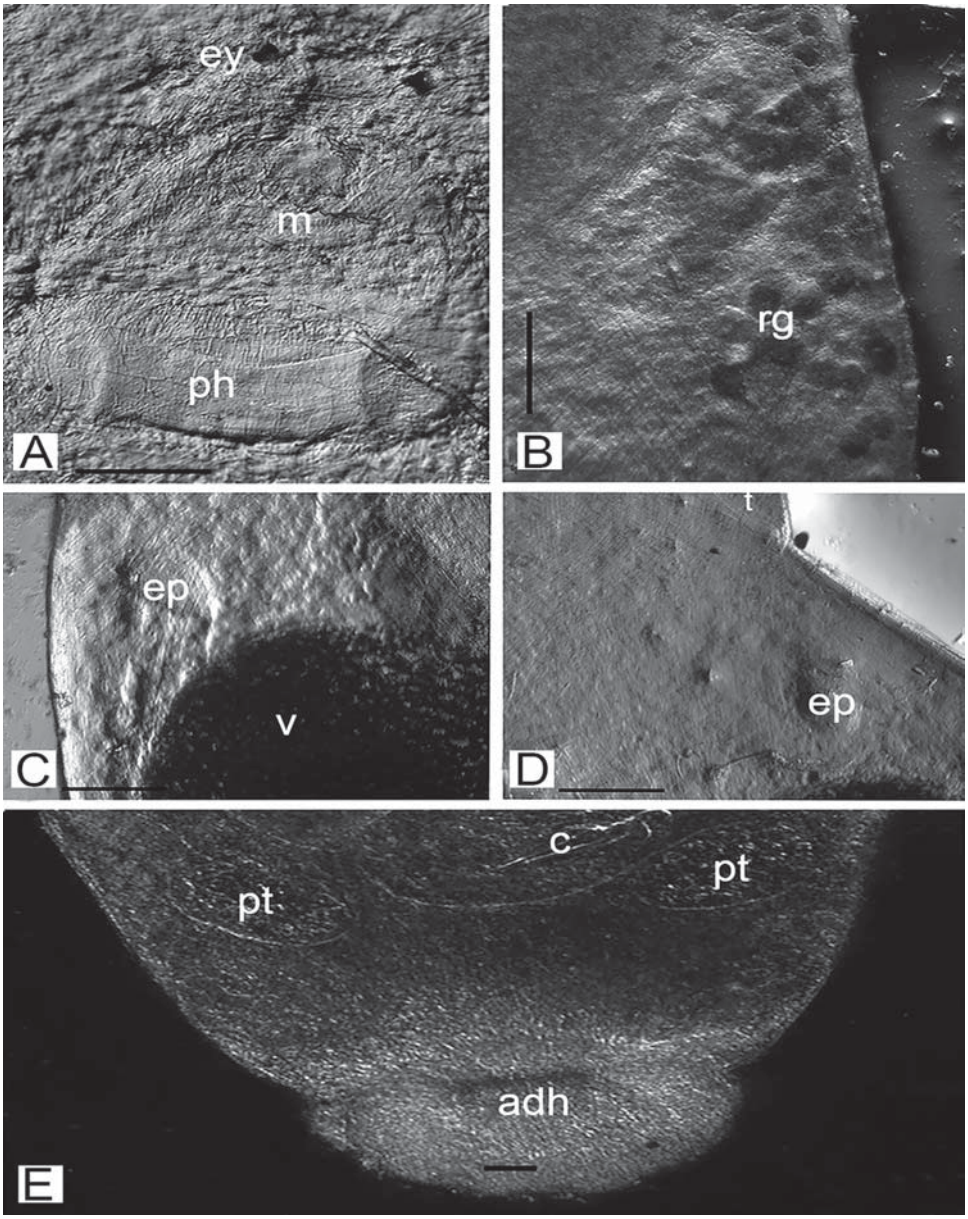

Fig. 4. Details of organization of *Temnosewellia semperi* (Weber, 1890); light microscopy. A — Details of eyes, mouth and the pharynx; B — rhabditogenic glands on trhe lateral sides of body; рабдитогенные железы в латеральной области тела; C, D — dorsolateral excretory syncytial plates (DLSPs) along with excretory pores; E — posterior region along with adhesive disc.

Abbreviations: adh — adhesive disc; c — cirrus; ep — excretory pore; ey — eye; m — mouth; ph — pharynx; pt — posterior testes; rg — rhabditogenic glands; v — vitellarium. Scale bars: A–D — 100 μm; E — 90 μm.

Рис. 4. Детали строения *Temnosewellia semperi* (Weber, 1890); световая микроскопия. A — Детали строения глаз, рта и глотки; B — рабдитогенные железы в латеральной области тела; C, D — дорсолатеральные экскреторные синцитиальные пластинки (DLSP) вместе с экскреторными порами; E — задняя область вместе с прикрепительным диском.

Обозначения: adh — прикрепительный диск; c — циррус; ep — экскреторная пора; ey — глаз; m — рот; ph — глотка; pt — задний семенник; rg — рабдитогенные железы; v — желточник. Масштаб: A–D — 100 μm; E — 90 μm.

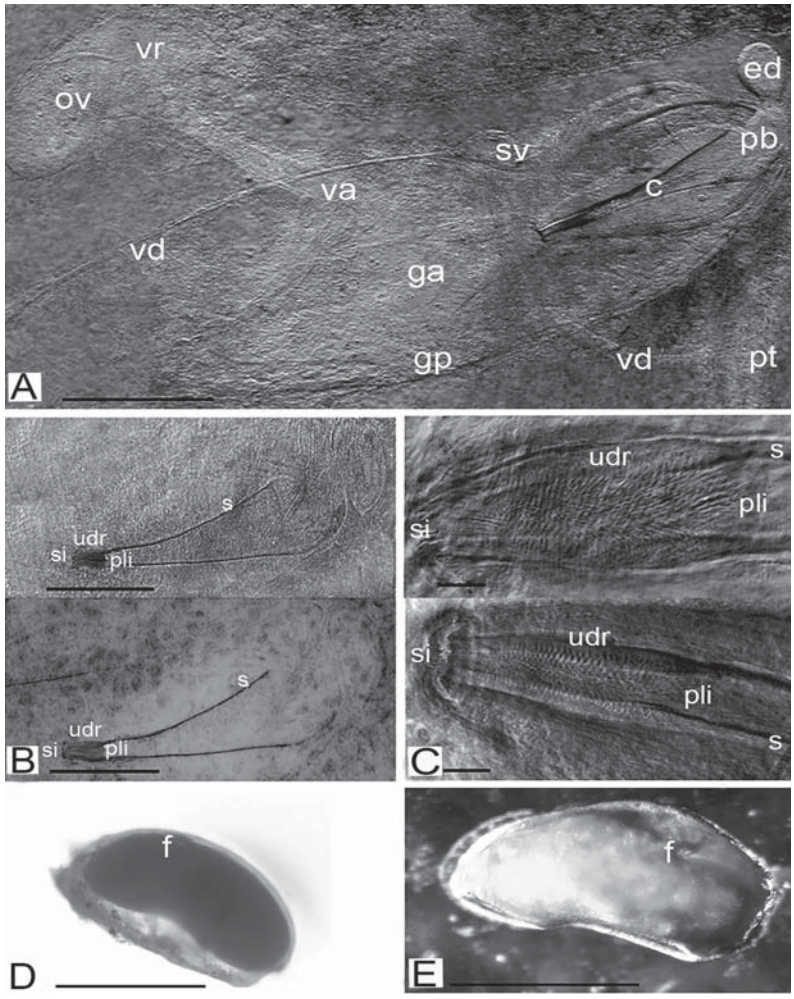

Fig. 5. Details of the reproductive system of *Temnosewellia semperi* (Weber, 1890). A — female reproductive system, consisting of ovary, vesicula resorbens and vagina and male reproductive system, consisting of posterior testes, vasa deferentia, seminal vesicle, ejaculatory duct, prostatic bulb, cirrus, genital atrium and genital pore; B–C — cirrus introvert observed in different focusing planes, showing spined introvert, unspined distal region, proximal limit of introvert and a prominent shaft; D–E — egg capsules with the filament bounded by opercular plates.

Abbreviations: c — cirrus; ed — ejaculatory duct; f — filament; ga — genital atrium; gp — genital pore; ov — ovary; pb — prostatic bulb; pli — proximal limit of introvert; s — shaft; si — spined introvert; sv — seminal vesicle; udr — unspined distal region; va — vagina; vd — vasa deferentia; vr — vesicula resorbens. Scale bars: A — 150 μm; B — 100 μm; C — 10 μm; D–E — 150 μm.

Рис. 5. Детали строения репродуктивной системы *Temnosewellia semperi* (Weber, 1890). A — женская репродуктивная система, состоящая из яичника, vesicula resorbens и вагины; мужская репродуктивная система, состоящая из задних тестикул, vasa deferentia, семенных пузырьков, эякуляторного канала, бульбы простаты, цирруса, полового атриума и генитальной поры; B–C — ввернутый циррус при различной фокусировке; показана ввернутая вооруженная часть, невооруженная дистальная область, проксимальная граница и развитый стержень; D–E — яйцевая капсула с филаментом вокруг оперкулярной пластинки.

Обозначения: c — циррус; ed — эякуляторный канал; f — филамент; ga — половой атриум; gp — половая пора; ov — яичник; pb — бульба простаты; pli — proximal limit of introvert; s — стержень; si — вооруженная часть; sv — семенной пузырёк; udr — невооруженная дистальная область; va — вагина; vd — vasa deferentia; vr — vesicula resorbens. Масштаб: A — 150 μm; B — 100 μm; C — 10 μm; D–E — 150 μm.

very long time. The specimens described in the present study however show similarities with the previous specimens reported by Weber (1890) who described the presence of five tentacles and brown pigmentation on the dorsal surface. Based on the presence of brown body pigmentation and five tentacles, the specimens therefore belong to the genus *Temnosewellia* (Damborenea, Cannon, 2001). The pigmentation which is more intense in adult forms and mainly on the dorsal region is a characteristic feature of *Temnosewellia*. Black pigmentation confined to the eyes observed in the present study is similar to *Temnosewellia vietnamensis* (Damborenea, Brusa, 2009) and *T. rouxii* (Merton, 1914). The elliptical excretory syncytial plates show a similar orientation as in the previous study made by Weber (1890) where they are located on the dorsolateral sides of the body just near the eyes. Like in some temnosewellids, the eggs are yellow, without peduncle and are cemented in a cluster directly on the host. The yellow eggs were also reported by Weber (1890), who described the colour of the eggs to be yellowish-brown that becomes dark brown during further development. The feature considered of relevant systematic value for species identification is the cirrus (Sewell *et al*., 2006). In this context, the present study shows that the specimen has nearly straight cirrus with the distal part of the introvert 54 μm long slightly dilated possessing numerous spines and a prominent distal unspined region which is similar to the previous study by Weber (1890) in which he described an urn-shaped spined introvert. Therefore this study provides a new insight into the occurrence of *Temnosewellia semperi* on the host *Maydelliathelphusa lugubris* found in the Umjajew freshwater stream of Meghalaya, India.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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