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Copulatory behaviour, copulation process and cocoon biology in earthworms–A review

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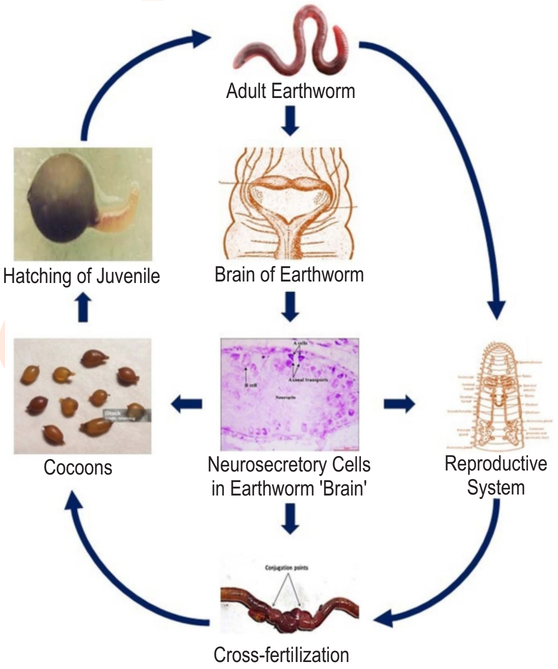
Abstract

Earthworms, despite having both female and male reproductive systems in the same individual (hermaphrodite), generally practice cross-fertilization. This is due to the fact that in earthworms, the testis matures earlier than the ovary. So, they generally do not undergo self-fertilization. The occurrence of parthenogenesis is not rare in earthworms. Probable involvement of extensive courtship behaviour involving repeated short touches with sensory prostomium of the copulating partners was recorded in Lumbricidae. During conjugation, the partners lie close together with their ventral body parts, keeping their heads in opposite directions. During sperm transfer, the spermathecal aperture of one individual makes intimate contact with the male genital aperture of the corresponding partner.

The mucus secretion from the clitellar section covers the opposing partners. Copulation lasts for about one hour. The worms move backwards, drawing the 'slime secretion tube' over the head. When the earthworms are entirely free, the ends of the tube close to form the cocoon. The shape of the cocoon is genus-specific. But its development time varies with species.This paper aims to review the process of copulation, cocoon formation, morphology, breeding strategies, neurosecretory control of copulation and cocoon production in earthworms.

Key words: Cocoon formation, Earthworms, Hermaphroditism, Neurosecretory cell, Parthenogenesis, Reproductive strategy



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Introduction

Earthworms are hermaphrodite animals because they contain functional male and female reproductive organs in a single individual. The male components comprise the testes lodged in testis sacs, testicular funnels (in most cases, there are two pairs in the 10th and 11th segments and singularly one pair in the 11th segment), seminal vesicles of varying numbers (2 - 4 in segments 9-12), vas deferens, male pores, prostate glands etc. The female components include ovaries (1 pair in the 13th segment), ovarian funnels in the 14th segment, and spermathecae (variable position and number). Anatomy of the reproductive system of Indian earthworm, *Pheretima posthuma* (Megascolecidae) and *Eutyphoeus gammiei* (Octochaetidae) have been described by Bahl (1927) and Chaudhuri and Gonchaudhuri (1997) (Fig. 1a, b) respectively. Earthworm species generally practice cross-fertilization between the partners with few exceptions (Edwards and Bohlen, 1996). The best examples of this phenomenon are found in European earthworms *Eisenia fetida* and *Lumbricus terrestris* and Indian earthworm *Eutyphoeus waltoni.* Dominguez *et al.* (2003) reported self-fertilization in the epigeic earthworm *Eisenia andrei,* where during sperm transfer, the worm bends itself in such a way that its spermathecal apertures make intimate contact with the male genital apertures on the ventral part of the clitellum of the same individual. Despite hermaphroditism, self-fertilization is prevented due to relative position of their male and female genital apertures and reproductive structures, and earthworms are protandrous.

Surface feeding, phytophagous epigeic species (*Eisenia* sp.) live above the surface. Phytogeophagous anecic earthworm *Lumbricus terrestris* lives in a vertical burrow and comes above the surface at night during feeding. According to Cosin *et al.* (2011), conjugation generally occurs above the soils in epigeic and anecic earthworms. Geophagous endogeic species such as *Drawida assamensis, Kanchuria* sp1, *Polypheretima elongata, Octochaetona beatrix* and *Pontoscolex corethrurus* live in horizontal complicated burrow systems. Presumably, copulation in them takes place inside the soils. Size assortative mating was reported in the epigeic earthworms *E. fetida* (Monroy *et al.,* 2005) and anecic Lumbricus terrestris (Novo *et al.,* 2010). Most earthworm species mate periodically throughout the year if the environmental conditions are suitable for reproduction (Edwards and Arancon, 2022).

Nuutinen and Butt (1997) used PVC cylinders (6.5 x 95 cm) and plastic boxes (15 x 19 x 12 cm) with field top soils for premating behavioural studies in *Lumbricus terrestris.* An infrared video camera recorded behavioural events under laboratory conditions (temperature 16 – 23°C). Koene *et al.* (2005) used small jars containing several centimetres of moist soil with frozen lettuce as feed for copulation studies in *Lumbricus terrestris.* Infrared-sensitive cameras were used to record the copulatory events. Bhattacharjee and Chaudhuri (2002) used earthen pots with suitable food (cow dung for phytophagous and soils for geophagous species) for studying cocoon production, morphology, incubation period, hatching patterns, and fecundity in Indian earthworm species viz. *Perionyx excavatus* Perrier, *Lampito mauritii* Kinberg, *Polypheretima elongata* (Perrier), *Pontoscolex corethrurus* (Muller), *Eutyphoeus gammiei* (Beddard), *Dichogaster modiglianii* (Rosa), and *Drawida nepalensis* Michaelsen under laboratory conditions. They reported a significant ($p < 0.05$) positive correlation between the number of hatchlings per cocoon and the incubation period in *Lampito mauritii.* Chaudhuri and Bhattacharjee (2011) studied the reproductive biology of eight tropical earthworms of rubber plantation, including *Drawida assamensis* Stephenson, *Octochaetona beatrix* Gates, *Metaphire houlleti* (Perrier), *Drawida papillifer papillifer* Gates, *Eutyphoeus comillahnus* Michaelsen, and *Dichogaster affinis* Michaelsen.

The possibility of uniparental reproduction indicating parthenogenesis was reported in peregrine species such as *P. corethrurus, D. affinis* and *O. beatrix* was reported by them. The same authors further reported a significant ($p < 0.05$) positive linear relationship between the incubation period (development time) and the length of earthworms. The temperature requirements for cocoon incubation vary among earthworm species was also reiterated by Chaudhuri and Bhattacharjee (2011). Thus, with an increase in temperature, the incubation period increased in *E. comillahnus* and *O. beatrix* and decreased in *D. affinis, M. houlleti* and *D. assamensis* within a temperature range of 20°C - 30°C under laboratory conditions. The incubation period was calculated as the time interval from the day of cocoon collection until the appearance of the first hatchling, plus half the time interval between cocoon collection and the previous inspection of food media (Butt, 1997). Recently, Bhattacharjee and Chaudhuri (2020) discussed the comparative cocoon biology of 16 different species of Indian earthworms where they concluded that the fecundity (power of reproduction in terms of cocoon or juvenile production of one species in a particular period of time) of peregrine species like *P. excavatus, P. corethrurus, P. elongata, D. affinis, D. modiglianii, D. bolaui, O. beatrix* is high and cocoon production is almost continuous throughout the year whereas fecundity is much lower in native species like *E. gammiei* and *E. comillahnus.*

Cocoon plays a major role in the continuity of life cycle of all earthworm species. With short life cycles, *Dichogaster bolaui* and *Dichogaster modigliani*i lay cocoons inside the soils, which remain dormant; following rain, the cocoons emerge as 'baby worms'. Cocoons also aid the persistence of earthworm populations through adverse climatic periods like winter (Gorres *et al.,* 2018). This is achieved through adaptive features like the development of frost-hardy cocoon castings in *Amynthus agretis, A. tokioensis, Metaphire hilgendorfi* and by prolonging the incubation or development periods (Nouri-Aiin and Gorres, 2019), thus creating a 'cocoon bank'. Neurosecretion refers to the secretion (neurohormone) elaborated by the neurosecretory cells which are specialised nerve cells differentiated in the direction of gland cells in the central nervous system of invertebrates. The functional activities of Earthworms are mainly controlled by neurosecretion (Chaudhuri, 2022). Debrained earthworms neither undergo copulation nor they produce any cocoon. The fact that earthworms with brains exhibit distinct secretory cycles in the cerebral type A and type B NSCs during pre-conjugating, conjugating and post-conjugating conditions (Banik *et al.*, 2020) indicates the role of NSCs (source of neurohormone) in the act of their sexual congress. The appearance of type A NSCs in the regenerated brain of debrained worm and the peak of type A neurosecretory cell activity coinciding with the peak of cocoon production during monsoon clearly indicates the possible role of cerebral type A NSCs in cocoon laying (Chaudhuri and Datta, 2020).

Copulatory behaviour: Olive and Clark (1978) suggested that chemical cues are a natural process of finding and attracting a partner in earthworms. Extensive courtship displayed during copulation was reported in *L. terrestris* by Nuutinen and Butt (1997), where the individual visits its neighbour's burrow and repeats short touches with the prostomium of the neighbouring mate. Copulatory behaviour followed by reproduction occurs repeatedly in *L. terrestris* during mating season, probably with different partners (Michiels *et al.,* 2001). Such prostomial touching behaviour before conjugation was also noticed under laboratory conditions in *E. fetida* by Banik *et al.* (2020). Earlier, Wallwork (1983) reported that prostomium is a "sensory lobe" in earthworms with many sensory receptor cells.

In Indian earthworms, *Eutyphoeus waltoni* Bahl (1927) observed their copulation in the early morning within five minutes after emergence from soils, with the closely apposed condition in a typical head-to-tail position. No prostomial touching behaviour was noticed in *Eutyphoeus waltoni* prior to conjugation. During copulation, while individuals of *E. waltoni* were intimately connected at their spermathecal and penial regions and closely applied to each other in the intervening area, the seven anterior segments of both worms were free. In the conjugating pair, Bahl (1927) observed feeding and conjugation going side by side.

Method of copulation: The method of copulation is not identical for all species (Edwards and Arancon, 2022). In the surface living, phytophagous (epigeic) earthworms, *Eudrilus eugeniae* and *E. fetida,* partners of each species come to lie together with their ventral body parts keeping their heads in opposing directions (Fig. 2a,b) with profuse mucus secretion from the mucus-secreting cells present in the epidermis. Banik *et al.* (2020) reported a rapid rise in the secretory activity of epidermal mucus-secreting cells in the conjugating earthworms of *E. fetida* compared to non-conjugating ones. Their bodies' ventral surface was grasped with setae and viscous mucus that covered both the individuals to tie them together for approximately one hour. Such binding during conjugation is strong enough to last for about 3 hrs in *L. terrestris* (Michiels *et al.,* 2001), which is aided by specialized setae to pierce the partner's skin, introducing a "bioactive substance" called allohormones (Koene and Ter Maat, 2001) from the setal gland which may increase the male reproductive success in the act of copulation. Such strong copulatory setae emerging from the male genital region are also found in endemic vertical burrowing geophagous (endo-anecic) earthworm, *Eutyphoeus gammiei* during breeding season for copulation (Chaudhuri and Gonchaudhuri, 1997). During conjugation in the lumbricid earthworms, the body is surrounded by a slime tube between the spermathecal region and the posterior border of the clitellum. Two slime tubes of the copulating worms remain in close contact, but are independent (Edwards and Arancon, 2022). In *L. terrestris,* a seminal groove extends from the male pore to the clitellum. Each seminal groove is a depression of the outer body wall formed as a series of pits following a contraction of the "archiform muscles" of the longitudinal muscle layer in the body wall. These muscles contract successively, beginning at the 15^{th} segment, and each contraction forms a pit. These pits carry seminal fluid as droplets from the male genital aperture towards the clitellum, where it eventually enters the spermathecae of the conjugating partner (Edwards and Bohlen, 1996).

According to Bahl (1927) method of copulation in the Indian anecic earthworm, *Eutyphoeus waltoni* at the soil surface was entirely different from that of the *Lumbricus species. E. waltoni* mate in the early morning (before 9 am) and after sunset at 6.30 pm. Bahl (1927) described the process as simple and direct, with no involvement of clitellum and seminal groove to transfer seminal fluid inside the spermathecae. Copulatory papillae in different species of *Eutyphoeus* have an important role during copulation. These papillae are of two types. The permanent papillae are circular cup-shaped depressions found in the inter-segmental grooves on the ventral surface. The temporary papillae are found as conical outgrowths of the body wall along the segments' mid-line. At the time of copulation, the temporary papillae fit into the permanent cup-shaped depressions of the opposing partner (Bahl, 1927). In them, the male pores open at the distal ends of two 'penial lobes' or 'penes' on segment 17, and these penes, along with the penial setae, with the aid of sphincter muscle, are inserted into spermathecal pores of co-operating worms during copulation. The exchange of sperm is mutual. The penes act as actual intromittent organs, injecting both spermatic and prostatic fluids into the spermathecae.

The prostate gland is present in all species of *Eutyphoeus.* The prostatic fluid acts as a nutrient medium for sperm, and sperm transference occurs without losing seminal fluid (Bahl, 1927). Due to this reason, according to Bahl (1927), tubular envelopes, as found in Lumbricidae, are not formed during "sexual congress" in *Eutyphoeus*. The occurrence of the intromittent organ as penes in *E. waltoni, E. gigas* and *E. incommodus* was also reported by Bahl (1927), indicating a similar copulatory mechanism in these Octochaetid worms.

Individuals of *E. fetida* were also seen to grip tightly and release each other several times under laboratory conditions (Banik *et al.,* 2020). Such movements may assist the entry of the seminal fluid containing spermatozoa into the sperm receptacle, *i.e.* spermatheca, a part of the female reproductive system.

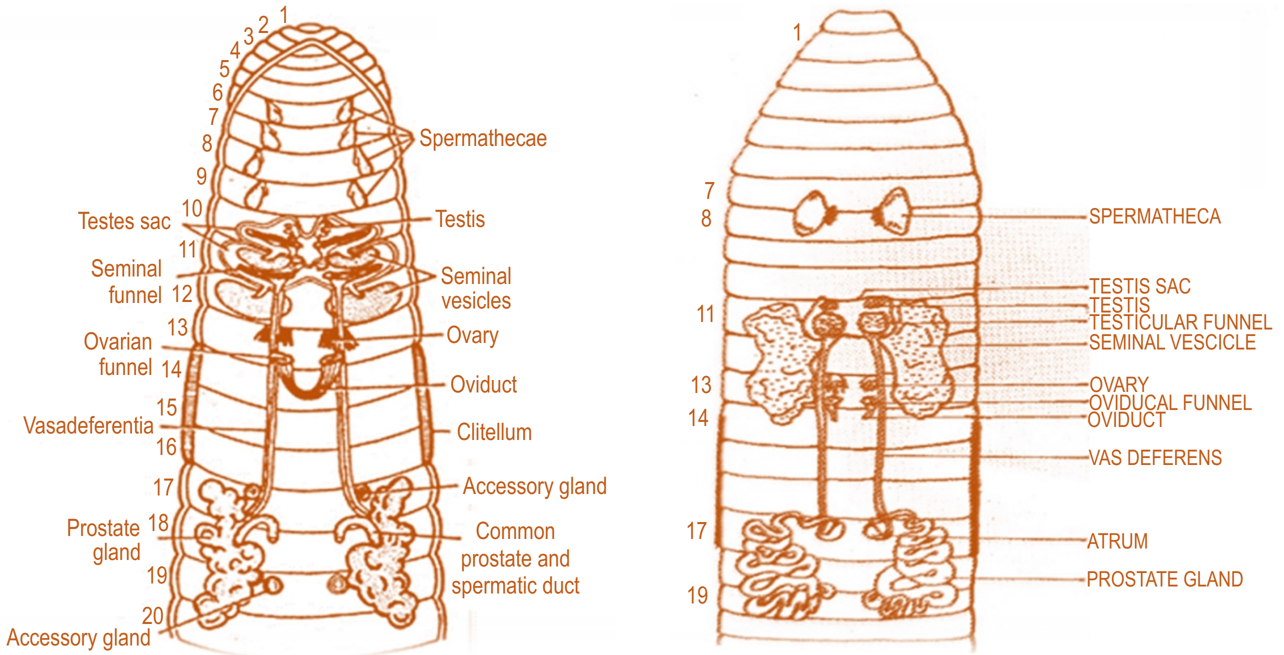

Fig. 1: Reproductive system of (a) *Metaphire posthuma* (Megascolecidae) (After Bahl, 1927) (b) *Eutyphoeus gammiei* (Octochaetidae) (After Chaudhuri and Gonchaudhuri, 1997).

Fig. 2: Act of Copulation in (a) *Eudrilus eugeniae* and (b) *Eisenia fetida* (After Banik *et al.,* 2020).

Without copulatory setae in earthworms, the sperm recipient may control the take-up and release sperms due to the presence of muscle fibres in the spermatheca wall (Breidenbach, 2002). Manipulation of copulatory setae or, in their absence, the activity of muscle fibres in the spermatheca wall is supposed to be helpful for the sperm donor because it increases the chances of fertilization (Koene *et al.,* 2005). In *Pheretima* sp., where 3 to 4 pairs of spermathecae are present, Tembe and Dubesh (1961) reported that during sexual congress, the male pores first come in contact with the hindmost pair of spermathecal apertures and discharge seminal and prostatic fluid into them. In fact, before fertilization, the cocoon is formed outside the clitellum. Then, each worm peristaltically moves backwards, and the seminal fluid is discharged into the next pair of spermatheca until all of them are filled. During backward movement, earthworms pass over female genital pores, where eggs are discharged inside the cocoon, where fertilization takes place to form a zygote. Thus, fertilization is generally external in most earthworm species (Edwards and Bohlen, 1996). According to Sims and Gerard (1985), the numbers of spermathecae differ between species. For example, both *E. gammiei* and *Microscolex phosphoreus* have one pair of spermatheca, *L. terrestris* 2 pairs, *Allolobophora chlorotica* 3 pairs, *Amynthas corticis* and *Metaphire posthuma* 4 pairs whereas *Polypheretima elongata* possesses more than 4

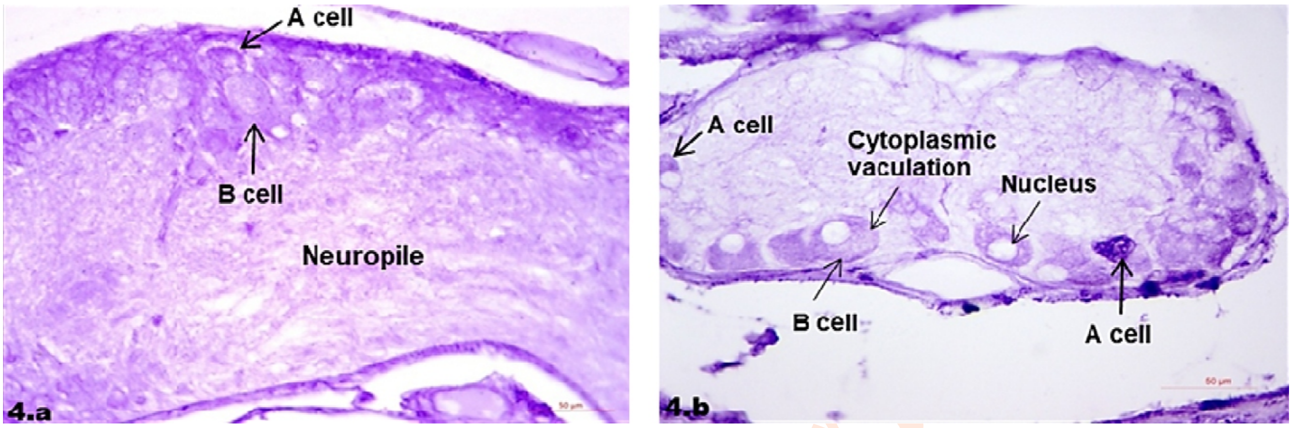

Fig. 3: Cyto-morphic changes in the Neuro-secretory cells (Type A and B cells) of *Eisenia fetida* during conjugation (After Banik *et al.,* 2020).

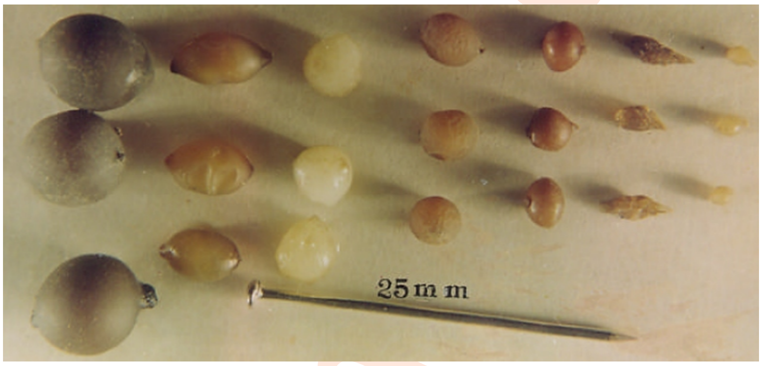

Fig. 4: Cocoons of different tropical earthworm species (Left to right: *Eutyphoeus gammiei, Lampito mauritii, Pontoscolex corethrurus, Polypheretima elongata, Drawida nepalensis, Perionyx excavatus* and *Dichogaster modiglianii*) (After Bhattacharjee and Chaudhuri, 2002).

pairs of spermathecae (Gates, 1972). According to Koene *et al.* (2005), these differences in the numbers of spermathecae may result from an evolutionary competitive race between species for sperm storage.

In Octochaetid earthworms such as *E. waltoni, E. gigas* and *E. incommodus,* male pores are present on raised papillae armed with a pair of penial setae lodged in a depression (seminal groove) on the ventral margin of clitellum (Julka, 1993). The papilla and the penial setae protrude during the act of sexual congregation and are inserted into the spermathecal duct of the corresponding partner through spermathecal pores (Bahl, 1927). The presence of genital papilla with penial setae present in a ventral depression in the posterior part of clitellum called seminal groove in *Eutyphoeus gammiei* (Chaudhuri and Gonchaudhuri, 1997) indicates that almost similar mechanism of sperm transfer may also occur in this species. In *Eutyphoeus waltoni,* copulation occurs above the soil surface, the detailed mechanism of which was earlier described by Bahl (1927). The eudrilid earthworm *Schubotziella dunguensis,* has a single median male pore formed by the fusion of two copulatory pouches and a single median spermatheca with the external opening. During copulation, the male pores on the copulatory pouch turn outwards and are inserted into the spermathecal opening (Edwards and Bohlen, 1996).

According to Edwards and Arancon (2022), the earthworm species which transfer the spermatozoa by direct methods, *i.e.,* discharge of male gametes directly to spermathecal opening do not form mucous tubes as observed in the lumbricid earthworms. During copulation, worms keep them close by possessing 6-10 shorter, thicker, and less curved setae in the anterior pre-clitellar region. The short setae flex inwards and grip the partner, while the long, pointed and grooved setae on the ventral side of the clitellum pierce the body wall of the opposing worm (Edwards and Arancon, 2022). Copulation continues for about 45 minutes in *Eudrilus eugeniae* and one hour in *Eisenia fetida* under laboratory conditions (Banik *et al.,* 2020). It is presumed that in one instance of successful conjugation from a pair of earthworm species, two cocoons (one from each individual) are produced. Noticeably, unlike the unfertilized one, a fertilized cocoon always submerges in the water. Recently, Banik *et al.* (2020) suggested the involvement of the cerebral and ventral ganglionic neurohormone in copulation in *Eisenia fetida* because it was found that "de-brained" earthworms did not undergo conjugation. However, the worms with "brain" conjugated with the partner displayed cellular changes and secretory dynamics in the cerebral and ventral ganglionic neurosecretory cells, type A and type B in pre-conjugating, conjugating and post-conjugating earthworms. These results clearly indicate the probable involvement of cerebral and ventral ganglionic neurosecretion released from peptide-secreting Type A cells and amine-secreting Type B cells in the phenomenon of conjugation in earthworms, in general, and *E. fetida* in particular (Banik *et al.,* 2020) (Fig 3a, b).

Formation of cocoon: The clitellum of the conjugating worms produces a secretion that eventually hardens over its outer surface (Banik *et al.,* 2020). When the slime tube becomes sufficiently rigid, the worms move backwards, thus drawing the mucus tube over its head. Later, when the mates become entirely free, the terminal ends of the tube close to form cocoons. The cocoon contains a nutritive albuminous fluid produced by the clitellar gland cells, the ova and spermatozoa, which are discharged into it when the tube moves over the spermathecal openings. Cocoons continue to form until all the stored seminal fluids in the spermathecae are used up. The role of cerebral neurohormone secreted by Type A cells during cocoon production in earthworms has recently been reported in *Perionyx ceylanensis* by Chaudhuri (2022) and Chaudhuri and Datta (2021). The appearance of well-differentiated deep stained Type A NSCs in the regenerated brain of cephalic amputated earthworm *P. ceylanensis* and the peak in activity of Type A NSC as revealed by a rise in the number of cerebral Type A cells along with axonal transport, cytoplasmic vacuolations and increase in their nucleocytoplasmic indices during both summer and monsoon (the pick activity period in earthworms) indicates utilisation of neurosecretory material and also hyperactivity of NSCs during cocoon production. The lowest nucleocytoplasmic indices and low content of neurosecretory materials of cerebral Type A cells of *P. ceylanensis* reveal their lowest neurosecretory activity to generate fewer cocoons, recorded in the winter brain (Chaudhuri and Datta, 2021). Thus, seasonal fluctuations in the activity of Type A cells coincided with the seasonal variation of cocoon production in earthworms. This also reveals the possible role of cerebral Type A cells in cocoon production. The cell volume, nuclear volume and nucleocytoplasmic ratio were significantly higher ($p < 0.05$) in cerebral neurosecretory Type A cells in *P. ceylanensis* during summer and monsoon than in winter (Chaudhuri and Datta, 2021). A positive and significant ($p < 0.05$) relationship between nucleocytoplasmic indices with room temperature ($r = 0.41$) and cocoon production ($r = 0.42$) indicates that cocoon generation in earthworms is influenced by temperature and the nucleocytoplasmic indices of cerebral Type A cells (Chaudhuri and Datta, 2021). Abiotic factors like light, soil moisture, temperature (Chaudhuri and Datta, 2020), quality of diet (Datta and Chaudhuri, 2020) and biological factors viz. age, sexual maturity (Parthasarathi and Ranganathan, 2000) and earthworm biomass (Okrzesik *et al.,* 2013; Chaudhuri and Datta, 2020) are also other important factors that influence cerebral NSCs for cocoon production. Hagadorn (1962) and Jadhav *et al.* (2001) emphasised the importance of cerebral Type A cell's neurosecretion for controlling the reproductive cycles in the leech, *Theromyzon rude* (Hagadorn, 1962) and male crab, *Uca lactea annulipes* (Jadhav *et al.,* 2001).

Cocoon morphology: Cocoon is roughly oval-shaped but has a typical shape for each species (Bhattacharjee and Chaudhuri, 2002; Chaudhuri and Bhattacharjee, 2011; Debnath and Chaudhuri, 2020) (Fig. 4). Thus, the cocoon may be spindle-shaped (*P. excavatus*), oval (*M. posthuma, L. mauritii*), spheroidal (*Polypheretima elongata, Pontoscolex corethrurus, Eutyphoeus gammiei, E. comillahnus, Octochaetona beatrix, Lenogaster chittagongensis*), pear-shaped (*Dichogaster modiglianii, D. bolaui),* onion-shaped (*Drawida nepalensis, D. assamensis, D. papillifer papillifer*) and irregular in outline (*Metaphire houlleti*) (Bhattacharjee and Chaudhuri, 2020). In our view, the shape of the cocoons in earthworms is genus-specific rather than species-specific as advocated by Edwards and Bohlen (1996). The freshly laid cocoons are generally opaque and turn bright brown over time with the appearance of vascularization. The colour of the cocoons probably varies with its incubation period. They may be dark straw (*Perionyx excavatus, P. ceylanensis*), light straw (*Lampito mauritii, Dichogaster bolaui*), straw colour (*Lenogaster chittagongensis*), light yellow (*Metaphire houlleti, M. posthuma, Dichogaster affinis*), dark grey (*Eutyphoeus gammiei, E. comillahanus*), olive green (*Octochaetona beatrix*), reddish (*Drawida nepalensis*), dark brown (*D. assamensis*), light brown (*D. papillifer papillifer*) in colour. The cocoon is soft when first formed but becomes harder in contact with air and resistant to damage and drying. Scanning electron microscopic studies on cocoons of three earthworm species, *P. excavatus, P. ceylanencis* and *E. eugeniae* (Chaudhuri and Datta, 2020), revealed that a significant part of the cocoon body wall had fibrils of different dimensions, except for its two terminal ends (Fig. 5).

Energy dispersion X-ray Spectroscopy studies on elemental analysis of cocoon wall confirmed the presence of various elements viz. C, N, O, Na, Mg, Al, Si, P, S, K, Ca, F, etc. (Chaudhuri and Datta, 2020). The colour of the cocoon varies from whitish (when formed) to yellow, greenish, or brownish and differs significantly in size and shape. As cocoons are derived from clitellum, generally, the cocoon dimension correlates with the size of the clitellum. Thus, the giant Australian earthworm, *Megascolides australis,* produces the largest cocoons, which may measure up to 75 × 20 mm. While the *L. terrestris* (similar in size to *Metaphire posthuma*) produces 6 × 5 mm cocoons. The largest (*Eutyphoeus gammiei*) and smallest (*Dichogaster affinis*) earthworm species from Tripura, North-east India produce the largest (diameter 7.4 × 6.8 mm, fresh weight 103 mg) and smallest (2 × 1 mm, 1.87 mg) spherical cocoons (Bhattacharjee and Chaudhuri, 2002). Later, Chaudhuri and Datta (2020) found a significant ($P<0.01$) positive correlation between the diameter of

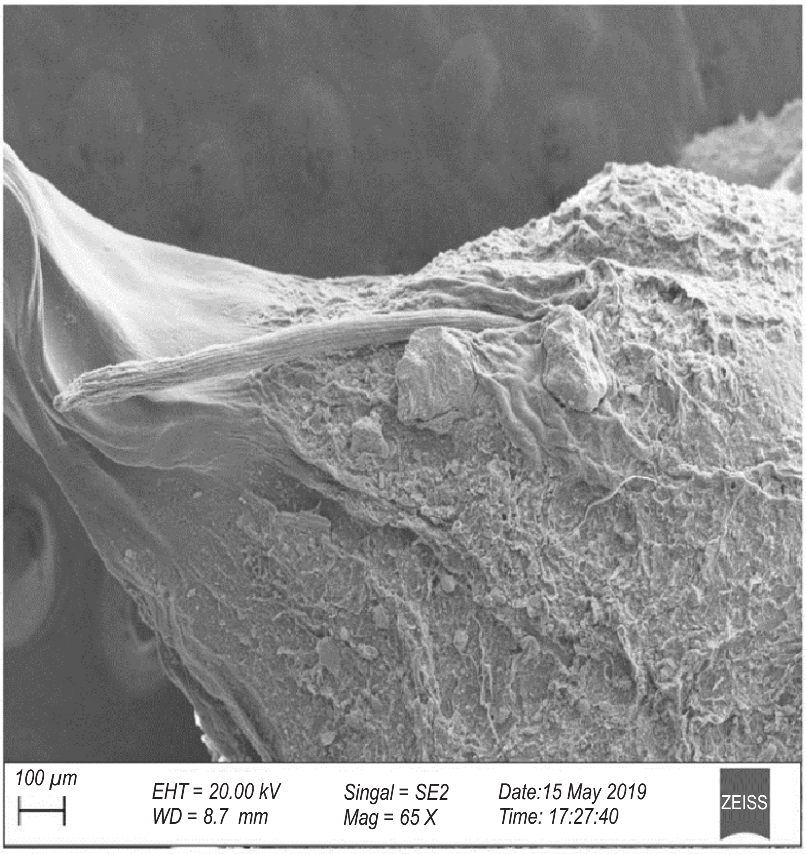

Fig. 5: SEM photograph of the lateral wall of the cocoon in *Eudrilus eugeniae* showing the abundance of fibrils (After Chaudhuri and Datta, 2020).

the clitellum and that of cocoon, and also the length of the clitellum and that of cocoon in *P. excavatus, P. ceylanensis* and *E. eugeniae,* respectively. Senapati and Sahu (1993) found a positive relationship between the size of the adult and the size of the cocoon in earthworms. Recently, a detailed account of the cocoon biology of 16 North-east Indian earthworm species has been discussed by Bhattacharjee and Chaudhuri (2020). Cocoons of earthworm species possess ornamentations, which are probably their ecological adaptations. Thus, cocoons possess a circle of bristles at one end and an elongated pointed end at the other (*Dichogaster modiglianii, D. bolaui, D. affinis*), two small curved/hook-like ends at terminal sides (*Polypheretima elongata*), short pointed structures at both the terminal ends (*Eutyphoeus comillahnus*), well-developed protrusions/ pointed ends at both the ends (*Drawida nepalensis, D. assamensis, D. papillifer papillifer*), pointed spine like structure at both the ends (*Metaphire posthuma, M. houlleti*), short protrusions at both the ends (*Octochaetona beatrix*). Ornamentations are absent in *Lampito mauritii, Pontoscolex corethrurus, Lenogaster chittagongensis,* whereas in the phytophagous vermicomposting earthworm species like *P. excavatus, P. ceylanensis* and *E. eugeniae,* cocoons are armed with tufts of stiff hairy structures at the pointed end and a broad funnel-shaped structure at the opposite ends (Chaudhuri and Datta, 2020). The appearance of these bristles seemed to be the adaptive features of surface-dwelling epigeic earthworm species that enable the cocoons to adhere to the litter in the surroundings (Chaudhuri and Bhattacharjee, 2002). The "baby worms" generally emerge through the broader end with a 'crown' shaped structure (Fig. 6) having less concentration of silicon and a high concentration of oxygen and nitrogen, as revealed by recent SEM observations on cocoons of *P. excavatus, P. ceylanensis* and *E. eugeniae* (Chaudhuri and Datta, 2020).

Reproductive strategies, fecundity and hatching success: Earthworms are semi-continuous or continuous breeders producing eggs most of the time in a year (Olive and Clark, 1978), depending on the moisture levels of soils. Examples of earthworms with continuous breeding strategies (cocoon production throughout the year) are *P. excavatus, P. corethrurus, D. modiglianii, D. affinis, O. beatrix, P. elongata,* while earthworm species like *L. mauritii* are semi-continuous and *E. gammiei* and *E. comillahnus* are discrete breeders (Bhattacharjee and Chaudhuri, 2002; Chaudhuri and Bhattacharjee, 2011; Bhattacharjee and Chaudhuri, 2020). In moist soils, earthworms deposit their cocoons near the surface and place them much deeper if the soil is dry. Most earthworm species produce cocoons when soil moisture, temperature, food (organic matter) supplies,

Fig. 6: Emergence of *Perionyx ceylanensis* juvenile from the cocoon through the broad crown-shaped terminal end (After Chaudhuri and Datta, 2020).

and other environmental factors (pH, aeration) are favourable. Within the temperature range of 25 to 31°C, the growth, reproduction, and cocoon production occur in tropical earthworms (Chaudhuri and Debnath, 2020). Temperature beyond the optimum level acts as a cue for neurosecretory activity, thus affecting cocoon production (Olive and Clark, 1978). Moisture is the limiting factor for earthworm survival. Dung or compost-dwelling earthworms require excess moisture conditions than the soil-dwelling species. Thus, soil moisture requirement for cocoon production in *D. nepalensis, P. elongata* and *L. mauritii* was within a range of 28% - 42% (Bhattacharjee and Chaudhuri, 2002), while bedding moisture of 70% – 80% was required for *P. excavatus, E. eugeniae* and *E. fetida* (Chaudhuri and Debnath, 2020).

The kind and amount of food available not only influence the size of earthworm populations but also the species present and their growth rate and reproduction (Edwards and Arancon, 2022). Earlier, Evans and Guild (1948) showed that more cocoons were produced when the worm was provided with decaying animal organic matter than those provided with fresh plant material. They further reiterated that earthworms fed on any nitrogen-rich diets grew faster and produced more cocoons than those with little nitrogen available. The appearance of biomass peak earlier than the reproductive peak and lowering of biomass value followed by a decline in the juvenile population of *Perionyx excavatus* in various experimental diets (cow dung alone and its mixtures with straw, bamboo leaf litter or kitchen waste) was earlier reported by Chaudhuri and Bhattacharjee (2002) that probably indicates that earthworms begin reproduction after attainment of a certain level of biomass. The same author reported the highest and lowest reproduction rates through cocoon production in the cow dung-straw and cow dung-kitchen waste diet mixtures, respectively, in *P. excavatus*. In geophagous earthworm *P. corethrurus,* Nath and Chaudhuri (2014) reported nutritional superiority (in respect of organic carbon% and total nitrogen%) of soil-cow manure-rubber leaf, soil-cow manure-rubber saw-dust and soil-cow manure-bamboo leaf littermixture over the cow dung diet to attain sexual maturity (as indicated by clitellum development) followed by increase in fecundity. The same authors found the appearance of biomass peak earlier than the reproductive peak in the treatments irrespective of the types of diet, which probably indicates that earthworms begin their reproduction after attainment of a certain level of biomass. Huerta *et al.* (2005) reported enhanced reproduction of tropical earthworm *Polypheretima elongata* after adding *Zea mays* and *Mucuna pruriens* into earthworm cultures in the laboratory. The highest individual fresh weight (5.8 g after 63 days) and the highest cocoon production (33 cocoons per individual in 98 days) were obtained when a litter of *Mucuna pruriens* var. *utilis* were added to the soil. In India, a good number of cocoons are laid from the end of February to July, when the temperature rises, and the greatest number of cocoons are produced during the monsoon and post-monsoon periods.

The number of earthworm cocoons produced in a season differs greatly depending on the species and climate. Satchell (1967) advocated a direct correlation between the number of cocoons generated by any species and how much this species is exposed to adverse environmental factors such as temperature, extreme desiccation, predation pressure, etc. Thus, epigeic species *E. fetida*, *E. eugeniae*, *P. excavatus*, *D. modiglianii*, *D. affinis*, etc., exposed to environmental hazards tend to produce more cocoons to enable them to survive in surface soils. These species living in deeper soil layers with predictable environments (*L. terrestris, E. gammiei*) produce fewer cocoons. Cocoon production/worm/year (fecundity) of *P. excavatus, P. corethrurus, D. modiglianii, Lampito mauritii, Drawida nepalensis, Polypheretima elongata* and *E. gammiei* was 156, 118, 68, 43, 29, 23 and 1 (Bhattacharjee and Chaudhuri, 2002). Similarly, cocoon production/worm/year was 11.5, 10.8, 16.8, 2.2, 46.5 and 40 for *D. papillifer papillifer, D. assamensis, M. houlleti, E. comillahnus, D. affinis* and *Octochaetona beatrix,* respectively (Chaudhuri and Bhattacharjee, 2011). According to Debnath and Chaudhuri (2020), cocoon production/adult/year for the earthworm species inhabiting organic waste deposits, such as *P. excavatus, D. bolaui, L. mauritii* and *M. posthuma* (*Pheretima posthuma*) was 187, 92, 83 and 25, respectively. Cocoon production, cocoon development and hatching, all are critically influenced by the environmental temperature (Chaudhuri and Debnath, 2020).

The thermal threshold for the hatching of cocoons exists in the majority of species. The ability of earthworm embryos to develop within a particular temperature range should be considered as an adaptation to particular habitat condition, making it possible for the juveniles to hatch out when favourable environmental conditions arise (Holmstrup *et al.,* 1991; Debnath and Chaudhuri, 2020). Since cocoons can resist desiccation and

Table 1: Culturing method used for each of the sixteen tropical earthworm species

Species	Collected from	Pot type	Pot size (ltr)	Soil used	Nutrient additive	Amount of soil (g)	Amount of additive (g)	Moisture content (%)
Perionyx excavatus	Dung deposit site	Earthen pot	4.5	Air dried and sieved pasture soil	Sieved Cowdung dust	600	100	70 - 80
Lampito mauritii	Pasture	Earthen pot	4.5	Air dried and sieved pasture soil	Sieved Cowdung dust	600	50	25 - 30
Polypheretima elongata	Pasture	Earthen pot	4.5	Air dried and sieved pasture soil	Sieved Cowdung dust	600	50	25 - 35
Pontoscolex corethrurus	Rubber plantation soil	Earthen pot	4.5	Air dried and sieved rubber plantation soil	Sieved Cowdung dust	2000	200	40
Eutyphoeus gammiei	Pasture	Earthen pot	4.5	Air dried and sieved pasture soil	Sieved Cowdung dust	4000	100	25 - 35
Eutyphoeus comillahnus	Rubber plantation soil	Earthen pot	4.5	Air dried and sieved rubber plantation soil	Sieved Cowdung dust	1000	100	40
Dichogaster modiglianii	Dung deposit site	Earthen pot	1	Air dried and sieved Garden soil	Sieved Cowdung dust	200	100	28 - 42
Dichogaster bolaui	Poultry litter sites	Earthen pot	1	Field soil	Sieved cowdung dust	500	50	50 - 60
Dichogaster affinis	Pasture and cowdung deposit site	Earthen pot	1	Air dried and sieved rubber plantation soil	Sieved cowdung dust	500	5	40
Drawida nepalensis	Pasture and cowdung deposit site	Earthen pot	4.5	Air dried and sieved pasture soil	Sieved cowdung dust	200	50	25 - 35
Drawida assamensis	Rubber plantation soil	Earthen pot	4.5	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000	200	40
D. papillifer papillifer	Rubber plantation soil	Earthen pot	4.5	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000	200	40
Metaphire houlleti	Rubber plantation soil	Earthen pot	4.5	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000	200	40
Metaphire posthuma	Sawdust dumping site	Earthen Pot	5	Field soil	Sieved cowdung dust	4000	400	20 - 30
Octochaetona beatrix	Rubber plantation soil	Earthen pot	4.5	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000	200	40
Lennogaster chittagongensis	Rubber plantation soil	Earthen pot	1	Air dried and sieved rubber plantation soil	Sieved cowdung dust	500	5	40

cold, they may enable the earthworm population to survive under adverse conditions. The zygote number in a cocoon ranges from 1 to 20 depending on the earthworm species belonging to family Lumbricidae. The incubation period of cocoon is the time gap between the day of cocoon laying and hatching. According to Edwards (1988), the average number of cocoons produced by *E. fetida* was 3.3 hatchlings per cocoon. Hatching percentage of cocoon was >50 in *P. corethrurus* (90%), *D. affinis* (81.5%), *D. modiglianii* (77.5%), *D. bolaui* (82%), *M. houlleti* (91.66%), *O. beatrix* (81%), *E. comillahnus* (63.6%), *L. mauritii* (70%), *P. excavatus* (52.5%), and <50 in *P. elongata* (40%), *D. nepalensis* (37.5%), *Metaphire posthuma* (25%), *E. gammiei* (20%), *D. papillifer papillifer* (11.5%), *D. assamensis* (11%) (Bhattacharjee and Chaudhuri, 2002; Chaudhuri and Bhattacharjee, 2011; Debnath and Chaudhuri, 2020). Although earthworms generally produce only one hatchling from a single cocoon, number of more than one hatchling from one earthworm cocoon (*D. assamensis* 1.30 ± 0.07, *M. houlleti* 1.23 ± 0.03, *L. mauritii* 1.67 ± 0.11, *P. elongata* 1.33 ± 0.12, *D. nepalensis* 1.8 ± 0.19, *P. excavatus* 1.15 ± 0.08, *D. bolaui* 1.7 ± 0.05) are also in the records (Bhattacharjee *et al.*, 2014; Debnath and Chaudhuri, 2020). Production of >1 earthworm hatchling from a single cocoon perhaps shortens the development time of cocoons due to fast utilization of limited resources by the developing embryos (Debnath and Chaudhuri, 2020).

Higher fecundity with high cocoon-hatching success in *Pontoscolex corethrurus, Dichogaster modiglianii, Dichogaster affinis, Metaphire houlleti, Octochaetona beatrix* seem to be the most important adaptive features of these exotic species of earthworms for their successful colonization with worldwide distribution, and, also their possible utilization in vermiculture based biotechnology. Despite being a top soil species, the low rate of cocoon production with low hatching success in *Drawida papillifer papillifer* and *D. assamensis* is probably related to their endemicity (Chaudhuri and Bhattacharjee, 2011). The culture process and biological characteristics of sixteen different tropical

Table 2: Biological characteristics of cocoons and hatchlings of sixteen tropicalearthworm species

Parameters	***Dichogaster affinis***	***Drawida nepalensis***	***Drawida assamensis***	***D. papillifer***	***Metaphire houlleti***	***Metaphire posthuma***	***Octochaetona beatrix***	***Lennogaster chittagongensis***
Adult size (mm)	40-70 x 2-3	80-120 x 4-5	60-80 x 3-4	45 - 90 x 4-5	100-160 x 3-6	80-140 x 3-8	60 - 120 x 4-5	50-130 x 4-6
Cocoons	(n=20)	(n=40)	(n=20)	(n=20)	(n=20)	(n=40)	(n=20)	(n=3)
i) Shape	i) Spheroidal	i) Onion shaped	i) Onion shaped	i) Onion shaped	i) Irregular oval	i) Oval	i) Spheroidal	i) Spheroidal
ii) Length(mm)	ii) 2.05 ± 0.05	ii) 4.6 ± 0.21	ii) 4.02 ± 0.26	ii) 2.9 ± 0.04	ii) 3.0 ± 0.07	ii) 7.1±0.5	ii) 4.35 ± 0.13	ii) 1.07 ± 0.02
iii) Breadth(mm)	iii) 1.05 ± 0.05	iii) 3.0 ± 0.28	iii) 2.68 ± 0.21	iii) 1.98 ± 0.06	iii) 2.4 ± 0.12	iii) 3.1±0.1	iii) 3.6 ± 0.14	iii) 1.01 ± 0.05
iv) Colour	iv) Light yellow	iv) Reddish	iv) Dark Brown	iv) Light brown	iv) Light yellow	iv) Light yellowish	iv) Olive green	iv) Straw colour
v)FreshWeight(mg)	v) 2.07 ± 0.13	v) 14.4 ± 0.5	v) 18.10 ±0.58	v) 17.3 ± 1.11	v) 13.41 ± 0.62	v) 26.1±0.41	v) 34.81 ± 1.54	v) 1.87 ± 0.23
vi) Ornamentation	vi) Elongated pointed end on one side and circlet of bristles on the other	vi) Well developed pointed end on either side	vi) Protrusions at both ends	vi) Protrusions at both ends	vi) Pointed spine like structure at one end	vi) Both ends bear small spines	vi) Short protrusions at both ends	vi) Absent
Frequency of cocoon production	Continuous	Semi-continuous	Semi - continuous	Semi - continuous	Semi - continuous	Discrete	Continuous	Discrete
Cocoon production worm-1 year-1	46.5	29	10.8	11.5	16.8	24	40	ND
Incubation period (days)	21.28 ± 1.27	24.26 ± 1.58	29.2 ±0.59	20.8 ± 0.55	64.6 ± 0.96	25.5±0.33	49.2 ± 1.90	ND
Hatching success (%)	81.51 (n=22)	37.50 (n = 15)	33.33 (n=108)	18.26 (n=115)	91.66 (n=168)	25	80.95 (n=400)	ND
Hatchlings cocoon-1	1	1.80 ± 0.19	1.30 ± 0.07	1	1.23 ± 0.03	1.0	1	ND
Hatchling size								
i. Length (mm)	i) 15.8 ± 0.32	i) 7.8 ± 0.76	i) 11.03 ± 0.79	i) 13.1 ± 0.52	i) 16.2 ± 0.72	i) 5.1±0.23	i) 26.6 ± 0.84	ND
ii. Breadth (mm)	ii) 1.0 ± 0.00	ii) 1.4 ± 0.21	ii) 1.02 ± 0.02	ii) 1.0 ± 0.00	ii) 1.0 ± 0.00	ii) 1.0±0.00	ii) 1.0 ± 0.00	
iii. Weight (mg)	iii) 5.10 ± 1.08	iii) ND	iii) ND	iii) 12.27±0.63	iii) 11.0 ± 0.74	iii) ND	iii) 46.5 ± 1.17	
	(n=20)	(n=5)	(n=20)	(n=20)	(n-20)	(n=10)	(n=20)	
Room temperature during incubation (°C)	26.19 ± 1.53	30.35 ± 0.24	20.60 ± 0.48	24.84 ± 0.95	25.41 ± 0.11	28±0.54	24.56 ± 0.48	ND
Room temperature during hatching (°C)	26.33 ± 1.41	29.56 ± 0.60	28.75 ± 0.85	28.75 ± 0.85	30.25 ± 1.05	29.02±0.11	21.83 ± 0.26	ND

ND - Not Determined

Table 2. Contd

Parameters	***Perionyx excavatus***	***Lampito mauritii***	***Polypheretima elongata***	***Pontoscolex corethrurus***	***Eutyphoeus gammiei***	***Eutyphoeus comillahnus***	***Dichogaster modiglianii***	***Dichogaster bolaui***
Adult size (mm)	90-150x3-5	95-150x3-6	100-300x3-6	60-120x4-6	182-410x7-10	70-155x4-6	22-60x1-2	20-40x1-3
Cocoons	(n=40)	(n=40)	(n=40)	(n=40)	(n=5)	(n=20)	(n=40)	(n=50)
i) Shape	i) Spindle like	i) Oval	i) Spheroidal	i) Spheroidal	i) Spheroidal	i) Spheroidal	i) Pear shaped	i) Pear shaped
ii) Length (mm)	ii) 6.52 ± 0. 44	ii) 5.0 ± 0.4	ii) 3.6 ± 0.35	ii) 5.0 ± 0.28	ii) 7.4 ± 0.45	ii) 3.5 ± 0.22	ii) 2.0 ± 0.28	ii) 2.5± 0.1
iii) Breadth (mm)	iii) 2.1 ± 0.26	iii) 2.8 ± 0.17	iii) 2.8 ± 0.17	iii) 3.2 ± 0.33	iii) 6.8 ± 0.33	iii) 2.9 ± 0.23	iii) 1.3 ± 0.1	iii) 1.2±0.2
iv) Colour	iv) Dark straw	iv) Light straw	iv) Yellowish	iv) White	iv) Dark grey	iv) Dark Grey	iv) Off-white	iv) Light straw
v) Fresh Weight (mg)	v) 5.0 ± 0.4	v) 20.6 ± 0.8	v) 30.2 ± 1.8	v) 21.0 ± 0.6	v) 103.2 ± 1.8	v) 21.46 ± 3.2	v) 1.5 ± 0.04	v) 1.3±0.22
vi) Ornamentation	vi) Bristles at the pointed end	vi) Absent	vi) Two small curved pointed end	vi) Absent	vi) Absent	vi) Short pointed structure at both apices	vi) Bristle at one end and other end pointed	vi) Bristle at one end and other end pointed
Frequency of cocoon production	Continuous	Semi continuous	Continuous	Continuous	Discrete	Discrete	Continuous	Continuous
Cocoon production worm^{-1} year^{-1}	156	43	23	118	1	2.2	68	92
Incubation period (days)	12.8 ± 0.31	14.93 ± 0.51	49.53 ± 1.77	29.03 ± 1.40	110	51.21 ± 3.25	14.16 ± 0.48	14.2±0.6
Hatching success (%)	52.50 (n = 21)	60 (n = 31)	40 (n = 15)	85 (n = 33)	20 (n = 1)	63.64 (n=22)	77.50 (n = 31)	82 (n = 30)
Hatchlings cocoon^{-1}	1	1.67 ± 0.11	1.33 ± 0.12	1.03 ± 0.02	1	1	1	1.7±0.05
Hatchling size								
i) Length (mm)	i) 4.8 ± 0.52	i) 12.7 ± 1.6	i) 24.0 ± 1.5	i) 6.60 ± 0.45	i) 50	i) 25.1 ± 0.83	i) 5.8 ± 0.33	i) 4.23±0.4
ii) Breadth (mm)	ii) 1.0 ± 0.1	ii) 1.4 ± 0.21	ii) 1.8 ± 0.17	ii) 2.0 ± 0.44	ii) 3	ii) 1.1 ± 0.1	ii) 1.0 ± 0.0	ii) 1.00±0.0
iii) Weight (mg)	iii) ND (n = 5)	iii) ND (n=5)	iii) ND (n=5)	iii) ND (n=5)	iii) ND (n=5)	iii) ND (n=20)	iii) ND (n=5)	iii) ND (n=10)
Room temperature during incubation (°C)	31.07 ± 0.26	29.71 ± 0.27	30.38 ± 0.15	30.11 ± 0.16	30.46	26.75 ± 0.52	30.85 ± 0.14	28.47±0.52
Room temperature during hatching (°C)	29.52 ± 0.25	29.60 ± 0.49	30.61 ± 0.53	29.76 ± 0.34	28.0	22.14 ± 0.30	29.88 ± 0.31	29.4±0.53

ND - Not Determined

earthworm species under laboratory conditions are given in Table 1 and 2. The earthworm species *Lenogaster chittagongensis,* was cultured and maintained under almost similar conditions in field soils, but hatchlings never emerged from the cocoons. There may be unique demands for the hatching of cocoons in such a native earthworm species, which was not met under laboratory conditions (Bhattacharjee and Chaudhuri, 2020).

Asexual reproduction: Parthenogenesis: Some earthworm species exhibit parthenogenesis, a form of asexual reproduction where the young ones develop from the unfertilized eggs without any genetic contribution from the male gametes. One of the most important factors of parthenogenesis is the evidence of male sterility. This includes the retention of testes, seminal vesicles, and prostates in a juvenile form in adults and the absence of iridescence of male funnels, ducts and spermathecae (Edwards and Arancon, 2022). The phenomenon of parthenogenesis is not homogeneously distributed in earthworms. According to Cosin *et al.* (2011), parthenogenesis is found only in Lumbricids and Megascolecids and not found in Glossoscolecids. In contrast, Chaudhuri and Bhattacharjee (2011) recorded parthenogenesis in some Megascolecidae, Octochaetidae and Glossoscolecidae species. Thus, among eight earthworm species of rubber plantation in Tripura of North-east India, F1 juveniles of *P. corethrurus, D. affinis, M. houlleti* and *O. beatrix* maintained singly in pot cultures, following their maturation, produced fertile cocoons that showed successful juvenile emergence (Chaudhuri and Bhattacharjee, 2011). These juveniles showed normal growth and development under laboratory conditions.

This indicates that these earthworm species do not require partners for breeding, *i.e.,* they are parthenogenetic. So, in addition to regular cross-fertilization, some species of earthworms also reproduce by parthenogenesis. Except for *Eisenia andrei,* there is no record till date so far on the occurrence of self-fertilization in earthworms due to the fact that the testis always mature earlier than the ovary in earthworms. Occurrence of parthenogenesis in several other species, like *Microscolex dubius, Plutellus papillifer, P. umbellularia, Pontodrilus bermudensis, Pheretima alexandri, P. anomala, P. campanulata, Udeinaa vesicula, Pontosclex corethrurus, P. defecta, P. elongata, P. infantilis, P. varians, Eukerria hortensis, E. subandina, Malabaria levis, M. sulcata* etc. have recently been reported by Edwards and Arancon (2022). Earlier, Satchell (1967) reiterated that *Octolasium* sp., *Eiseniella* sp., *Aporrectodea rosea* and *Dendrobaena rubida* (Lumbricidae) usually reproduce parthenogenetically. According to Edwards and Bohlen (1996), in *Eisenia fetida,* while normal practice was to produce cocoons by cross-fertilization, they can also reproduce asexually by parthenogenesis. However, the number of cocoons produced by the former process is much more than that of the latter. Reproduction cannot occur without a clitellum, ovaries, oviducts and possibly ovisacs. However, the male organs are not essential. So, the absence in various degrees of male reproductive structures like testes, seminal vesicles, vas deferens, copulatory chambers, prostates, spermathecae, and genital or penial setae may indicate occasional or obligatory parthenogenesis in earthworms (Gates, 1972; Edwards and Arancon, 2022). Thus, parthenogenesis is one of the primary sources of morphological variability within the reproductive structures of earthworms. Most of the parthenogenetic earthworms are polyploid (Cousin, 2011). *Dendrobaena rubida* may be diploid, triploid, tetraploid, hexaploid or octoploid biotypes, revealing extremely high liability of the genetic system. One of the main advantages of polyploidy in parthenogenetic species is the increase in genetic variability, allowing them to adapt to various environmental conditions. It is not known whether parthenogenic earthworms may have arisen from the hybridization process. Future research will tell this.

In spite of hermaphroditism, earthworms practice cross-fertilization as their testis matures earlier than the ovary. Involvement of chemical cues with extensive courtship behaviour with repeated short touches by sensory prostomium among the partners during sexual congress are reported. Copulation takes place for about one hour. Sperm transfer occurs between the spermathecal apertures of one individual with the male genital apertures of the corresponding partner. Conjugation is followed by cocoon formation through the contribution of the clitellum. Fertilization takes place inside the cocoon from which after a development period, juvenile (one or more) emerges. Both conjugation and cocoon formation is under neurosecretory control. Shape of cocoon is genus specific but development time varies with species.

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