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Cocoon Biology of Sixteen Different Species of Indian Earthworms

Subhalaxmi Bhattacharjee* and Priyasankar Chaudhuri**

*Dept. of Zoology, Holy Cross College, West Tripura - 799210
**Corresponding author, Dept. of Zoology,
Tripura University, Suryamaninagar - 799022
drpsc1957@gmail.com

Abstract

Information on cocoon biology of sixteen different tropical earthworm species like *Pontoscolex corethrurus* (Műller), *Drawida assamensis* Stephenson, *Drawida papillifer papillifer* Gates, *Drawida nepalensis* Michaelsen, *Eutyphoeus comillahnus* Michaelsen, *Eutyphoeus gammiei* Beddard, *Metaphire houlleti* (Perrier), *Dichogaster affinis* Michaelsen, *Dichogaster bolaui* (Michaelsen), *Dichogaster modiglianii* Rosa, *Octochaetona beatrix* Gates, *Lennogaster chittagongensis* (Stephenson), *Metaphire posthuma* (Vailant), *Perionyx excavatus* Perrier, *Lampito mauritii* Kinberg, and *Polypheretima elongata* (Perrier) are presented in this paper. Detailed cocoon morphology is made available here. Fecundity of peregrine species like *P. excavatus, P. corethrurus, P. elongata, D affinis, D modiglianii, D. bolaui, O. beatrix* have been found to be high and cocoon production is also continuous in them. Whereas fecundity is much lower in native species like *E. gammiei, E. comillahnus.* A positive relationship between size of adult and cocoon size has also been found. Room temperature during incubation had been found to affect incubation period.

The more continuous and high rate of cocoon production and high hatching success in *P. corethrurus, O. beatrix, D. affinis, D. bolaui, D. modiglianii* approve their usage in vermiculture based technology. The geophagous species like *P. corethrurus and O. beatrix* can be used for improvement of quality of different land systems while phytogeophagous species can be made available for stabilization of organic wastes.

Keywords: cocoon, cocoon morphology, incubation period, fecundity, hatching success

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Introduction

The basic module of survival of any species on this planet is self-maintenance and species continuance.

Tripura, the third smallest state of India, is bestowed with a rich diversity of earthworms (Dhar and Chaudhuri, 2020, Chaudhuri and Nath, 2008, Chaudhuri and Nath, 2012, Chaudhuri and Chakraborty 2017, Chaudhuri and Jamatia, 2017). A total of 24 different earthworm species are described from different agricultural plantations alone.

Vermiculture has proven itself as a sustainable way to solve several waste management problems, as well as, in endeavours to increase productivity of various agricultural practices. Knowledge of different aspects of the reproductive strategies of earthworm species found in large numbers in native soils will definitely ensure better utilization of local earthworm fauna in various practices of vermitechnology.

Earthworms are very popular as protein feed for poultry, piggery and fishery (Akter *et al.*, 2020). They are an excellent source of high quality animal protein. But the natural population of earthworms is available on a seasonal basis only. Also, earthworms have high tendency of bioaccumulating hazardous residues of heavy metals, pesticides, antibiotics etc. in their tissues. Hence, there is a very high possibility of transferring these chemicals whenever earthworms are used as feed. Prevention of biomagnification of these toxic wastes is only possible through earthworm rearing with good soils with organic additives (cow dung).

The current study brings together the information on cocoon morphology, cocoon development, hatching success and fecundity of sixteen different earthworm species found in Tripura. Earthworms were collected from rubber plantation, municipal solid waste sites and pastures within West Tripura.

Earthworms are generally considered to be cross-fertilization hermaphrodites i.e. they receive and transfer sperms in the same copulation. In spite of hermaphroditism earthworms, in general, practice cross-fertilization due to the fact that their testes and ovaries mature at different times. And cases of self fertilization or parthenogenesis has also been known in them. The female components are ovaries, oviducal funnels, oviducts and spermathecae. The male reproductive components are testes, testicular sac, seminal vesicles, vasa differentia and prostate glands (Di'az Cosi'n *et al.,* 2011).

During copulation, spermatozoa from one partner reaches the spermatheca of the other partner and is stored. Cocoons are formed by secretions of clitellar glands of body wall. The stored sperms and ova from one partner is squeezed inside the cocoon and the outer covering gradually hardens in contact with air. The copulating pair of earthworms wriggle and gradually the cocoon is released.

Three ecological categories of earthworms such as Endogeic, Anecic and Epigeic forms are recognized. While fecundity is generally highest in Epigeic species, the rate of cocoon production is generally much lower in Endogeic and Anecic species.

Cocoons play a major role in the continuity of life cycle of all earthworm species. *Dichogaster bolaui, D. modiglianii* with short life cycle, lay cocoons inside soils which remain in dormant condition and following rain the cocoons emerge as ‘babyworms’. Cocoons even aid the persistence of earthworm populations through adverse climatic periods like winter (Görres *et al.,* 2018). This is achieved through adaptive features like development of frost- hardy cocoon casings in *Amynthus agretis, A. tokioensis, Metaphire hilgendorfi* and also by prolonging the hatching development periods (Nouri-aiin *et al.* 2019), thus creating a ‘cocoon bank’.

Methods

The climate of Tripura is broadly divided into 3 seasons: winter (November-February), summer (March-June) and monsoon (July-October).

For studying the reproductive strategies, a total of sixteen earthworm species were considered. Eight earthworm species viz. *Pontoscolex corethrurus* (Műller), *Drawida assamensis* Stephenson, *Drawida papillifer papillifer* Gates, *Eutyphoeus comillahnus* Michaelsen, *Metaphire houlleti* (Perrier), *Dichogaster affinis* Michaelsen, *Octochaetona beatrix* Gates, *Lennogaster chittagongensis* (Stephenson) were collected from the 25 year old rubber plantations at Mohanpur Block of Sadar subdivision (22°51´- 24°32´ N latitude and 90°10´- 92°21´E longitude) from August to October 2006 (Chaudhuri and Bhattacharjee, 2011).

Two earthworm species viz. *Dichogaster bolaui* (Michaelsen) and *Metaphire posthuma* (Vailant) which were found in municipal solid wastes sites in the West Tripura were collected during April 2018 (Chaudhuri and Debnath, 2020).

Six earthworm species viz *Perionyx excavatus* Perrier, *Lampito mauritii* Kinberg, *Polypheretima elongata, Drawida nepalensis* Michaelsen, *Dichogaster modiglianii* Rosaand *Eutyphoeus gammiei* Beddard were collected from pasture and dung deposit sites in Sadar subdivision of Tripura between July and October 1998 (Chaudhuri and Bhattacharjee, 2002). Adult earthworms were collected by means of digging (25cm x 25cm x 25cm) and hand sorting from their respective habitats by quadrat method (Chaudhuri and Bhattacharjee, 2011, Chaudhuri and Debnath, 2020). Worms were acclimatized in well-ventilated laboratory rooms for 1 month. Individual specimens were identified from the Zoological Survey of India, Solan.

Based on different field studies, the ecological categories, habit, habitat and size relationship of these earthworm species are presented in Table I.

Preparation of Culture Medium

The studies for reporting the reproductive behaviors of all the sixteen earthworm species were carried out under partially controlled laboratory conditions.

The size of earthen pot, within which earthworms were to be reared, was selected according to the general body length of each of the sixteen species of worms. Smaller worms like *Dichogaster bolaui, Dichogaster affinis* and *Lennogaster chittagongensis* were reared in earthen pots of small size. Medium sized worms were all accommodated in 4.5 lt pots.

All the pots received field soil. The soils were air dried, ground and sieved. Organic additive whenever added was 15 day old cowdung dust (particle size ≤ 2mm). Table III describes all the different types of culture media used.

The soil medium inside each pot was moistened daily with the use of hand spray on alternate days. Moisture level was maintained at about 40% in each of them. Only in case of *Perionyx excavatus* moisture was maintained at 70%. The moisture level of the food medium was measured periodically by gravimetric method.

One pair of healthy clitellate earthworm of the same species was introduced in each pot.

During the course of studies of three different years (Chaudhuri and Bhattacharjee, 2011, Chaudhuri and Debnath, 2020, Chaudhuri and Bhattacharjee, 2002) maximum and minimum room temperature was found to be (32.4°C in April and 19.6°C in

January) (Chaudhuri and Bhattacharjee, 2002),(32.18ºC in July and 20.05ºC in January) (Chaudhuri and Bhattacharjee, 2011) and (31.1ºC in May and 18.4ºC in January) (Chaudhuri and Debnath, 2020).

The room temperature was recorded daily using room thermometer. The maximum temperature was found to be around 31ºC and minimum temperature was found to be around 19ºC. All pots were maintained for about 1 year under laboratory conditions.

Whenever the death of worm was encountered, fresh pair of earthworm species with the same biomass were introduced in the pots. Five replicates were maintained for each species. The old culture media was replaced with a prescribed amount of fresh substrate at the end of each month, so that food was not a limiting factor for earthworm growth and reproduction.

Cocoon Studies

The contents of each pot were carefully examined on weekly basis using hand lens. The adult worms were separated from the culture media of each pot by hand sorting and weighed. The culture media was then gently washed by pouring water and cocoons were collected by sieving through 0.5 mm mesh sieve. The number of cocoons produced per individual was estimated. The size and weight of the cocoons were also measured. But before weighing, to get their actual weight, the cocoons were washed lightly in distilled water to remove debris adhering to their outer covering. Ornamentation and colouration of the fresh cocoons were also observed.

Incubation of Cocoons

The cocoons thus obtained were kept on moist filter papers spread over water soaked cotton (having 85% moisture). This arrangement was made inside a petri-dish having 15 cm diameter. One cocoon was kept in one dish only. The ten replicates were maintained for cocoon incubation of each species. Observations were made daily to record the external colour changes if any during the incubation period and also to determine the exact time of hatching. Mode of hatching and number of hatchlings emerging from each cocoon was also recorded. Fresh weight of new hatchlings, their size, site of emergence were recorded. The incubation period was calculated as the time interval from the day of cocoon collection until the appearance of first hatchling plus half the time interval between cocoon collection and previous inspection of food media which was 3.5 days in all the three case studies (Butt, 1993, Butt, 1997).

Statistical Analysis

The correlation between the mean room temperature and the incubation period for different earthworm species was tested by using simple regression analysis. Significant differences, if any, in cocoon production and three different seasons (summer, monsoon and winter) with respect to the room temperature for each earthworm species was compared by one way ANOVA followed by critical difference (CD) at 5% level of significance.

Results

Based on field studies the size, habitat, ecological category of sixteen different earthworm species are presented in Table I. These species have been described by Gates (Gates, 1972). Table II describes the characteristics and ecological categories of all the sixteen earthworm species studied. Table III describes the different culturing mediums used in every laboratory experiment. Table IV describes all the morphological characters of cocoons along with hatchling details of all the earthworm species.

Cocoons (Fig. 1 and Fig. 2) and newly emerged hatchlings of different earthworm species depict different characteristics.

Cocoons of different earthworm species studied in general are spheroidal in shape (Fig. 1) except in *Perionyx excavatus* and *M. houlleti* where cocoons are spindle shaped and irregular oval respectively. The elaborate pointed ends and bristles of cocoons of epigeic earthworms like *P. excavatus, D. affinis* and epianecic species like *M. houlleti* are the adaptive features of those species that enable them to adhere to the litter of the surroundings.

Fig. 1: Cocoons of earthworms (from left to right: *Eutyphoeus gammiei, Lampito mauritii, Pontoscolex corethrurus, Polypheretima elongata, Drawida nepalensis, Perionyx excavatus* and *Dichogaster modiglianii*)

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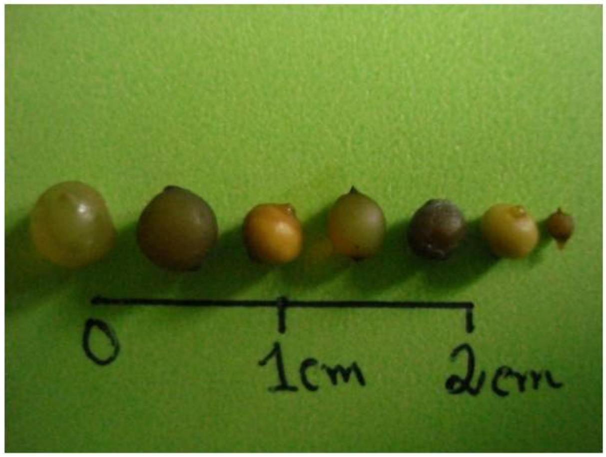

Fig. 2: Cocoons of *P. corethrurus, O. beatrix, D. assamensis, M. houlleti, E. comillahnus, D. papillifer papillifer* and *D. affinis* (from right to left).

Among the 16 species of earthworms studied, the largest cocoon was of the giant worm *E. gammiei* (diameter 7.4, fresh weight 103.2 mg) and the smallest cocoon was of *L. chittagongensis* (diameter 1.07 mm, fresh weight 1.87 mg). A positive relationship between the size of the adults and the size of cocoons produced by earthworms are on record (Lavelle, 1981, Senapati and Sahu, 1993). Freshly laid cocoons of most of the earthworm species were opaque, but those of *P. corethrurus* and *L. mauritii* were semi-transparent. The cocoon of *P. corethrurus* was pearly white in colour when freshly laid. The development time (incubation period) of cocoons varied among the earthworm species studied. The incubation period was found to be shortest in *P. excavatus* (12.8 days) and longest in *E. gammiei* (110 days). The incubation period was shorter in topsoil species like *D. modiglianii, D. affinis, D. papillifer papillifer, D. nepalensis, D. assamensis, P.corethrurus* and long in the subsoil species like *P. elongata, O. beartix, E. gammiei.*

Continuous breeding strategy was exhibited by most of the exotic peregrine species like *P. corethrurus, P. elongata, D. modiglianii, D. affinis, P. excavatus, D. bolaui, O. beatrix.* According to Chaudhuri and Datta (2020) native earthworm species *P. excavatus, P. ceylanensis, Eudrilus eugeniae* and exotic earthworms show continuous breeding strategies. They further reiterated that *P. excavatus* showed two peaks of cocoon production, one during March and another during September. *P. ceylanensis* had two peaks of cocoon production, one during September and another during

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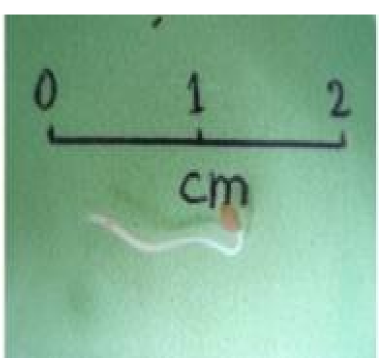
November. The *E. eugeniae* showed only one peak of cocoon production during March only. Semi-continuous breeding strategy was shown by most native peregrine species like *L. mauritii, D. nepalensis, D. assamensis, D. papillifer papillifer* and *M. houlleti.* Discrete breeding was shown by *E. gammiei, E. comillahnus* and *M. posthuma.* Within a certain range of temperature (22-35ºC), cocoon production increased significantly ($p < 0.05$) in *P. excavatus* and *P. caylaensis.* However, *E. eugeniae* showed reverse relationship (Chaudhuri and Datta 2020)

Recent SEM studies by Chaudhuri and Datta (2020) showed that emergence of juveniles occur through the broader terminal end with a crown shaped structure which is characterized by less concentration of silicon and high concentration of oxygen and nitrogen. In the middle zone of the cocoon, silicon remains as silicate (polymer) that inhibits the exit of juveniles. The porosityof the cocoon allows respiration of embryo inside (Edwards and Bohlem 1996).

(a) M. houlleti

(b) D. papillifer papillifer



(c) P. corethrurus

(d) D. affinis

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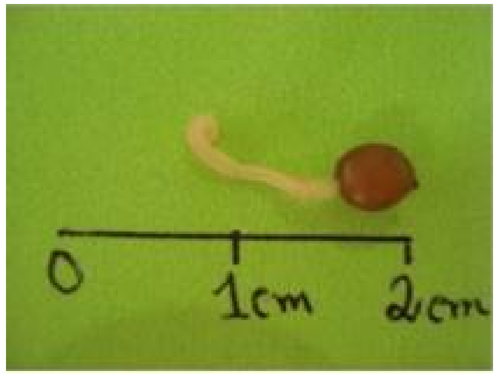

(e) O. beatrix

Fig. 3: Cocoons and newly emerged hatchlings of different earthworm species.

Values of total number of cocoons produced/adult/year varied with the species (**Table IV**). Bhattacharjee and Chaudhuri (2002) (Chaudhuri and Bhattacharjee, 2002) and Chaudhuri and Bhattacharjee (2011) (Chaudhuri and Bhattacharjee, 2011) reported the monthly variations of cocoon production for different earthworm species. There was significant increase ($p< 0.05$) in cocoon production during summer compared to winter in cases of *D. affinis, D. papillifer papillifer, D. assamensis, D. nepalensis, P. excavatus, P. corethrurus*. In many of the species under present study there was no significant difference in cocoon production during summer and monsoon. Temperature beyond optimum level act as a stimulus for decreased neurosecretory activity. This in turn negatively affect cocoon production (Olive and Clark, 1978).

A clear relationship was also found between fecundity and the location in soil profile for each earthworm species under study. The earthworm species which are generally located in deeper layers of the soil profile usually face lesser adverse conditions. Hence fecundity is low in them. Whereas earthworm species living towards the top layer of soil profile face environmental hazards and has very high fecundity. Hence, top layer resident earthworm varieties of Tripura like *P. corethrurus, P. excavatus, D. affinis, D. modiglianii, D. bolaui, L. mauritii, O. beatri*x have much higher fecundity than sub soil species like *E. comillahnus,* and *E. gammiei*.

The earthworm species, *L. chittagongensis* was cultured and maintained under almost similar conditions but hatchlings never emerged from the cocoons. This might be the unique demands for hatching of cocoons required in such native subsoil earthworm species was not met under laboratory conditions at that time.

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Discussion

There is a definite relationship between reproductive strategies and ecological categories in earthworm species of the tropics. Lavelle (Lavelle *et al.,*1998) designated these groups as **Group 1**: Large native endogeic and anecic species (16-32g fresh weight) with low fecundity (0.5-3.1 cocoons per adult per year) and 1 hatchling per cocoon. **Group 2**: medium sized species (1.2-6g) with intermediate fecundity (1.3 - 45 cocoons per adult per year); **Group 3**: small mainly polyhumic endogeic species (0.17-1.25 g fresh weight) and intermediate fecundity (10- 68 cocoons per adult per year) and usually one hatchling per cocoon. **Group 4**: generally small, mainly exotic and epigeic species (80- 550 mg fresh weight) with very high fecundity (50- 350 cocoons per adult per year). In this study it appears that, *E. gammiei* comes under Group 1: *E. comillahnus, P. elongata and O. beatrix* belongs to Group 2: *D. assamensis, D. papillifer papillifer, D. nepalensis, L. mauritii and M. houlleti* comes under Group 3: while *P. corethrurus, D. affinis, D. modiglianii, D. bolaui and P. excavatus* falls under Group 4.

Charles Darwin had described the intrinsic relations that the earthworms have with soil ecosystems (Darwin, 1882). Then Jones *et al.* had described them first as the 'ecosystem engineers (Jones, Lawton and Sachak, 1994). All these were attempts to describe earthworms as major contributors to the composition and functioning of soil ecosystem with varying species diversity. It may be considered that the reproductive strategy of these organisms are unique and also toughest of all the strategies for their survival.

Continuous breeding strategies with high fecundity and hatching success of the epigeic variety of worms and semi-continuous breeding strategy of anecic worms signifies their possible utilization in vermiculture based vermitechnology like vermicomposting and 'in-situ' earthworm technology for better crop growth.

Conclusion

Top soil earthworm species with high fecundity, short incubation period, high hatching success should be selected for vermiculture (culture of suitable top soil earthworm species in appropriate food substrate) and vermicomposting (organic waste management through synergistic action of epigeic earthworm and microbes). These processes are relevant in relation to in-situ (direct application of earthworm in soils) and ex-situ (vermicomposting) vermitechnology.

Recommendation

It is recommended to study its biology (especially growth and reproduction) before choosing the appropriate earthworm species for vermitechnology.

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Table I: Morphological Characteristics of Earthworm Species used for the study and their Ecological Categories

Sl. No.	Species	Family	Size (mm)	Pigmentation	Feeding Habit	Vertical Distribution (cm)	Ecological Category
1.	*Perionyx excavatus*	Megascolecidae	L 100–180 B 5–6	Deeply pigmented	Phytophagous	0–15	Epigeic
2.	*Lampito mauritii*	Megascolecidae	L 140–160 B 5–6	Lightly pigmented	Phytogeophagous	10–15	Top soil endogeic
3.	*Polypheretima elongata*	Megascolecidae	L 200–250 B 5–7	Unpigmented	Geophagous	30–45	Sub soil endogeic
4.	*Pontoscolex corethrurus*	Glossoscolecidae	L 70–90 B 4–5□□2	Lightly pigmented dorsally	Geophagous	10–15	Endogeic
5.	*Eutyphoeus gammiei*	Octochaetidae	L 200–400 B 7–10	Pigmented dorsally, lightly pigmented ventrally	Geophagous	15–45	Endogeic
6.	*Eutyphoeus commillahnus*	Octochaetidae	L=70-135 B=2-4	Lightly Pigmented	Geophagous	15-45	Endogeic
7.	*Dichogaster modiglianii*	Octochaetidae	L 25–40 B 2–3	Moderately pigmented	Phytogeophagous	0–10	Epigeic
8.	*Dichogaster bolaui*	Octochaetidae	L=25-35 B=1-3	Moderately pigmemted	Phytophagous/ Phytophagous	0-5	Epigeic/ Polyhumic Endogeic
9	*Dichogaster affinis*	Octochaetidae	L=35-42 B=1-2	Lightly Pigmented	Phytophagous	--	Epigeic

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Table I (Continuation): Morphological Characteristics of Earthworm Species used for the study and their Ecological Categories

Sl. No.	Species	Family	Size (mm)	Pigmentation	Feeding Habit	Vertical Distribution (cm)	Ecological Category
10	*Drawida nepalensis*	Moniligastridae	L 40–50 B 2–3	Lightly pigmented dorsally	Geophagous	10–15	Endogeic
11	*Drawida assamensis*	Moniligastridae	L=45-90 B=3-4	Deeply pigmented	Phytogeophagous	--	Epianecic
12	*Drawida papillifer papillifer*	Moniligastridae	L=45-90 B=3-4	Deeply pigmented	Phytogeophagous	0-5	Epianecic
13	*Metaphire houlleti*	Megascolecidae	L=100-160 B=3-6	Deeply Pigmented dorsally	Phytogeophagous	----	Epianecic
14	*Metaphire posthuma*	Megascolecidae	L=100-200 B=5-10	Lightly pigmented	Geophagous	5-15	Endogeic
15	*Octochaetona beatrix*	Octochaetidae	L=60-120 B=4-5	Lightly Pigmented	Geophagous	----	Subsoil Anecic
16	*Lennogaster chittagongensis*	Octochaetidae	L=35-42 B=1-2	Lightly Pigmented	Geophagous	--	Endogeic

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Table II: Earthworm Specimens used for the study and Physico-Chemical Conditions they were found during collection

Sl. No.	Species	Soil Organic Matter (g%)	Soil pH	Soil Moisture (g%)	Soil Temperature (ºC)	Ecological Category
1.	*Perionyx excavatus*	4.5-12	6.4-7.4	10-70	20-28	Epigeic
2	*Lampito mauritii*	0.5-4.5	5.8-7.2	10-40	20-28	Top soil endogeic
3.	*Polypheretima elongata*	2.5-4.5	6.9-7.2	10-40	20-28	Sub soil endogeic
4.	*Pontoscolex corethrurus*	1.40±0.12	4.68±0.06	22.97±0.74	25.85±0.19	Endogeic
5.	*Eutyphoeus gammiei*	2.05±0.51	4.77±0.05	25.99±2.07	25.30±0.10	Endogeic
6.	*Eutyphoeus commillahnus*	1.55±0.15	4.75±0.06	24.20±1.33	26.08±0.33	Endogeic
7.	*Dichogaster modiglianii*	2.5-8.5	5.7-7.2	10-60	20-28	Epigeic
8.	*Dichogaster bolaui*	3.68 2.39	5.38+0.74	28.86+11.26	25.67+0.48	Epigeic/ Polyhumic endogeic
9	*Dichogaster affinis*	4.84±3.63	5.70±1.17	32.70±18.34	25.20±0.20	Epigeic
10	*Drawida nepalensis*	1.59±0.06	4.88±0.07	27.40±3.48	25.45±0.005	Endogeic
11	*Drawida assamensis*	1.40±0.05	4.82±0.03	23.63±0.54	25.85±0.17	Epianecic
12	*Drawida papillifer papillifer*	1.22±0.05	4.64±0.07	23.55±0.75	26.77±0.15	Epianecic
13	*Metaphire houlleti*	1.55±0.26	4.80±0.09	23.47±0.90	25.90±0.25	Epianecic
14	*Metaphire posthuma*	3.9 ± 0.98 (1.2 - 6.2)	7.4 ± 1.1 (6 - 8)	23.91 ± 2.4 (18.8 - 31.0)	29.6 ± 0.86 (25.0 - 31.2)	Endogeic
15	*Octochaetona beatrix*	1.33±0.14.	4.22±0.01	21.80±1.10	27.25±0.25	Subsoil Anecic
16	*Lennogaster chittagongensis*	1.33±0.14	4.91±0.13	17.65±2.74	24.73±0.94	Endogeic

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Table III: Culturing Medium used for each of the sixteen species

Sl. No.	Species	Collected From	Pot Type	Pot Size	Soil Used	Nutrient Additive	Amount Of Soil	Amount Of Additive	Moisture Content
1	*Perionyx excavatus*	Dung deposit site	Earthen pot	4.5lt	Air dried and sieved pasture soil	Sieved cowdung	600gm	100gm	70 - 80%
2	*Lampito mauritii*	Pasture	Earthen pot	4.5lt	Air dried and sieved pasture soil	NO	600gm	0	25 - 30%
3	*Polypheretima elongata*	Pasture	Earthen pot	4.5lt	Air dried and sieved pasture soil	NO	600gm	0	25 - 35%
4	*Pontoscolex corethrurus*	Rubber plantation soil	Earthen pot	4.5lt	Air dried and sieved rubber plantation soil	Sieved cowdung	2000gm	200gm	40%
5	*Eutyphoeus gammiei*	Pasture	Earthen pot	4.5lt	Air dried and sieved pasture soil	NO	600gm	0	25 - 35%
6	*Eutyphoeus comillahnus*	Rubber plantation soil	Earthen pot	4.5lt	Air dried and sieved rubber plantation soil	Sieved cowdung	2000gm	200gm	40%
7	*Dichogaster modiglianii*	Dung deposit site	Earthen pot	4.5lt	Air dried and sieved Garden soil	Sieved cowdung	200gm	100gm	28 - 42%
8	*Dichogaster bolaui*	Poultry litter sites	Earthen pot	1lt	Field soil	Sieved cowdung dust	500gm	50gm	50 - 60%

Table III continuation.

Sl. No.	Species	Collected From	Pot Type	Pot Size	Soil Used	Nutrient Additive	Amount of Soil	Amount of Additive	Moisture Content
9	*Dichogaster affinis*	Pasture and cowdung deposit site	Earthen pot	1lt	Air dried and sieved rubber plantation soil	Sieved cowdung dust	500gm	5gm	40%
10	*Drawida nepalensis*	Pasture and cowdung deposit site	Earthen pot	4.5lt	Air dried and sieved pasture soil	NO	200gm	0	25 - 35%
11	*Drawida assamensis*	Rubber plantation soil	Earthen pot	4.5lt	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000gm	200gm	40%
12	*Drawida papillifer papillifer*	Rubber plantation soil	Earthen pot	4.5lt	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000gm	200gm	40%
13	*Metaphire houlleti*	Rubber plantation soil	Earthen pot	4.5lt	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000gm	200gm	40%
14	*Metaphire posthuma*	Sawdust dumping site	Earthen Pot	5lt	Field soil	Sieved cowdung dust	4000gm	100gm	20 - 30%
15	*Octochaetona beatrix*	Rubber plantation soil	Earthen pot	4.5lt	Air dried and sieved rubber plantation soil	Sieved cowdung dust	2000gm	200gm	40%
16	*Lennogaster chittagongensis*	Rubber plantation soil	Earthen pot	1lt	Air dried and sieved rubber plantation soil	Sieved cowdung dust	500gm	5gm	40%

Table IV: Biological features of cocoons and hatchlings of 16 different earthworm species of the tropics.

Parameter	1. *Perionyx excavatus*	2. *Lampito mauritii*	3. *Polypheretima elongata*	4. *Pontoscolex corethrurus*	5. *Eutyphoeus gammiei*	6. *Eutyphoeus comillahnus*	7. *Dichogaster modiglianii*	8. *Dichogaster bolaui*
Adult size (mm)	-------	-------	------	------	------	70-135 x 2.4	--------	-------
Cocoons i) Shape ii) Length(mm) iii) Breadth(mm) iv) Colour v) Fresh Weight(mg) vi) Ornamentation	(n=40) i) Spindle shaped ii) 6.52 ± 0. 44 iii) 2× 1 ± 0× 26 iv) Dark straw v) 5.0 ± 0.4 vi) Bristles at the pointed end	(n=40) i)Oval ii)5.0 ± 0.4 iii)2.8 ± 0.17 iv)Light straw v)20.6 ± 0.8 vi)Absent	(n=40) i)Spheroidal ii)3.6 ± 0.35 iii)2.8 ± 0.17 iv)Yellowish v)30.2 ± 1.8 vi)Two small curved pointed ends	(n=40) i) Spheroidal ii)5.0 ± 0.28 iii)3.2 ± 0.33 iv)White v)21.0 ± 0.6 vi)Absent	(n=5) i)Spheroidal ii)7.4 ± 0.45 iii)6.8 ± 0.33 iv)Dark grey v)103.2 ± 1.8 vi)Absent	(n=20) i) Spheroidal ii) 3.5 ± 0.22 iii) 2.9 ± 0.23 iv) Dark Grey v) 21.46 ±3.21 vi) Short pointed structure at both apices	(n=40) i)Pear shaped ii)2.0 ± 0.28 iii)1.3 ± 0.1 iv)Off-white v)1.5 ± 0.04 vi)One end with single pointed end and other bears a circlet of bristles	(n=50) i)Pear shaped ii)2.5± 0.1 iii)1.2±0.2 iv)Light straw v)1.3±0.22 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
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) ii) Breadth(mm) iii) Weight (mg)	i)4.8 ± 0.52 ii)1.0 ± 0.1 iii)------- (*n* = 5)	i)12.7 ± 1.6 ii)1.4 ± 0.21 iii)------ (n=5)	i)24.0 ± 1.5 ii)1.8 ± 0.17 iii)------ (n=5)	i)6.60 ± 0.45 ii)2.0 ± 0.44 iii)----- (n=5)	i)50 ii)3 iii)---- (n=5)	i)25.1 ± 0.83 ii)1.1 ± 0.1 iii)46.0 ± 3.41 (n=20)	i)5.8 ± 0.33 ii)1.0 ± 0.0 iii)------ (n=5)	i)4.23±0.4 ii)1.00±0.0 ii) ------ iii) (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

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Parameter	9. *Dichogaster affinis*	10. *Drawida nepalensis*	11. *Drawida assamensis*	12. *Drawida papillifer papillifer*	13. *Metaphire houlleti*	14.*Metaphire posthuma*	15.*Octochaetona beatrix*	16.*Lennogaster chittagongensis*
Adult size (mm)	60-120 x 4-5	---------	60-80 x 4-5	45 - 90 x 4-5	100-160 x 3-6	----------	60 - 120 x 4-5	50-130 x 4-6
Cocoons I. Shape II. Length(mm) III. Breadth(mm) IV. Colour V. Fresh Weight(mg) VI.Ornamentation	(n=20) I. Spheroidal II. 2.05 ± 0.05 III. 1.05 ± 0.05 IV. Light yellow V. 2.07 ± 0.13 VI. Elongated pointed end on one side and circlet of bristles on the other	(n=40) I.Onion shaped II.4.6 ± 0.21 III.3.0 ± 0.28 IV.Reddish V.14.4 ± 0.5 VI.Well developed pointed end on either side	(n=20) I. Onion shaped II. 4.02 ± 0.26 III. 2.68 ± 0.21 IV. Dark Brown V. 18.10 ±0.58 VI. Protrusions at both ends	(n=20) I. Onion shaped II. 2.9 ± 0.04 III. 1.98 ± 0.06 IV. Light Brown V. 17.30 ± 1.11 VI. Protrusions at both ends	(n=20) I. Irregular oval II. 30.0 ± 0.07 III. 2.4 ± 0.12 IV. Light yellow V. 13.41 ± 0.62 VI.Pointed spine like structure at one end.	(n=40) I.Oval II.7.1±0.5 III.3.1±0.1 IV.Light yellowish V.26.1±0.41 VI.Both ends bear small spines	(n=20) I. Spheroidal II. 4.35 ± 0.13 III. 3.6 ± 0.14 IV. Olive green V. 34.81 ±1.54 VI. Short protrusions at both ends	(n=3) I. Spheroidal II. 1.07 ± 0.02 III. 1.01 ± 0.05 IV. Straw colour V. 1.87 ± 0.23 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	-------
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	----------
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)	------
Hatchlings $cocoon^{-1}$	1	1.80 ± 0.19	1.30 ± 0.07	1	1.23 ± 0.03	1.0	1	---
Hatchling size I. Length (mm) II. Breadth(mm) III. Weight (mg)	I. 15.8 ±.0.32 II. 1.0 ± 0.00 III. 46.5 ± 1.17 (n=20)	I.7.8 ± 0.76 II.1.4 ± 0.21 III.------ (n=5)	I. 11.03 ± 0.79 II.1.02 ± 0.02 III.ND (n=20)	I.13.1 ± 0.52 II.1.0 ± 0.00 III.12.27 ± 0.63 (n=20)	I.16.2 ± 0.72 II.1.0 ± 0.00 III.11.0 ± 0.74 (n-20)	I.5.1±0.23 II. 1.0±0.00 III.------ (n=10)	I. 26.6 ± 0.84 II. 1.0 ± 0.00 III. 46.5 ± 1.17 (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	--------
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	----