39. Schwedenmann, L., Veldkamp, E., Brenes, T., O'Bien, J. and Backensen, J., Spatial and temporal variation in soil CO_2 efflux in an old-growth neotropical rainforest, La Selva, Costa Rica. *Biogeochemistry*, 2003, **64**, 111–128.
40. Luan, J., Liu, S., Zhu, X., Wang, J. and Liu, K., Role of biotic and abiotic variables in determining spatial variation of soil respiration in secondary oak and planted pine forest. *Soil Biol. Biochem.*, 2012, **44**, 143–150.
41. Yuko, Y., Hideaki, S., Yojiro, M. and Takayoshi, K., Effects of soil and vegetation types on soil respiration rate in larch plantation and a mature deciduous broadleaved forest in northern Japan. *Eurasian J. For. Res.*, 2006, **9-2**, 79–95.
42. Crill, P. M., Seasonal patterns of methane uptake and carbon dioxide release by temperate woodland soil. *Global Biogeochem. Cycles*, 1991, **5**, 319–334.
43. Takahashi, M. *et al.*, Soil respiration in different ages of teak plantations in Thailand. *Japan Agric. Res. Quatr.*, 2009, **43**, 337–343; http://www.jircas.affrc.go.jp

ACKNOWLEDGEMENTS. We thank CSIR, New Delhi for providing financial support under a research project. We also thank Prof. J. Srinivasan, IISc, Bangalore for constructive comments and input on the manuscript.

Received 5 March 2014; revised accepted 30 April 2014

Isolation of predominant bacterium from gut of earthworm *Lampito mauritii* for effective use in soil fertility

Sreejata Biswas[1,*], Pulak Lahiri[2,4] and Satadal Das[3]

[1]Department of Zoology, Bangabasi Morning College, 19, Rajkumar Chakraborty Sarani, Kolkata 700 009, India
[2]Formerly at Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700 019, India
[3]Peerless Hospital and B.K. Roy Research Centre, 360 Panchasayar, Kolkata 700 094, India
[4]Present address: 1 Ballygunge Place, Kolkata 700 019, India

Lampito mauritii **is an anecic earthworm living in the topsoil and it is geophytophagous in nature. This earthworm is an important soil macrofauna as it has the dual role of an 'ecosystem engineer' due to the ability to build burrows as well as 'keystone species' in soil food webs because of its function in degradation of organic wastes. The present study investigates the gut of this earthworm to find the most predominant bacterium harboured therein. Gut contents were regularly extracted and streaked on bacteriological media. The predominant type of colony was identified, isolated and streaked separately to get pure colonies. The microbe was subjected to several biochemical tests and also 16S rRNA sequencing for identification. On the basis of these tests, the bacterium was identified as** ***Bacillus cereus.*** **The microbe was used as a composting agent on solid wastes as a result of which good amount of plant nutrients, specially nitrogen (20.3 kg/acre), phosphate (27.4 kg/acre) and potassium (52.1 kg/acre) were found in the resultant manure. The compost thus obtained was then utilized for the production of vegetables with an attempt to protect soil environment, thus reducing the deleterious effects of chemical fertilizers.**

Keywords: Composting, gut bacteria, *Lampito mauritii*, organic waste, soil fertility.

THE living community of the soil, including both fauna and flora, plays a major role in decomposition, humification and litter formation[1]. Of the innumerable life forms that inhabit the soil, only a small number of macro invertebrates (earthworms, termites and ants) are distinguished by their capacity to excavate the soil and produce a wide variety of organomineral structures, such as excretions, nests, mounds, macropores, galleries and caverns. These organisms have been described as 'ecological engineers' of the soil[2] and their structures as 'biogenic structures'[3]. Earthworms form one of the major soil macrofauna to maintain dynamic equilibrium and regulate soil fertility[4]. The soil volume affected by earthworm activities is called the drilosphere[5], which is a major soil functional domain[6].

Earthworm activity does not only mediate macroaggregate formation, but also microaggregate formation[7,8]. Based on thin sections of the earthworm gut, casts and control soil from earthworm microcosms, several studies have shown that during gut transit organic materials are intimately mixed and become encrusted with the mucus to create new nuclei for microaggregate formation[7–9]. On the other hand, earthworm casts significantly affect plant growth through their effects on microorganisms, aggregation of soil and nutrient supply[10]. Casts have been shown to have enhanced microbial and enzyme activities and micro- and macro-nutrients[11]. Many authors have reported the occurrence of several species of bacteria and fungi in earthworm casts[12,13]. Many cellulolytic, nitrifying and denitrifying bacteria have been observed in earthworm casts[14]. Several workers have found that microorganisms flourish in earthworm casts. Teotia *et al.*[15] reported that earthworm casts had a bacterial count of 32.0 million/g compared to 6.0–9.0 million/g in the surrounding soil. Daniel and Anderson[16] experimented with *Lumbricus rubellus* and observed that the casts in four different soils contained greater number of bacteria than the soils. During formation in the earthworm gut, the 'would be' casts are colonized by microbes that begin to breakdown soil organic matter[17].

According to Julka *et al*[18], in India there are 590 species of earthworms with different ecological preferences. *Lampito mauritii* is the most widely distributed earthworm in

*For correspondence. (e-mail: sreejata26@yahoo.com)

different agro-ecosystems of India[19–24]. It is an anecic earthworm living in the topsoil and being geophytophagous, it consumes both litter as well as soil rich in organic matter. This earthworm is found to prefer decomposing grass of paddy (*Oryza sativa*) and finger millet (*Eleucine coracana*) to other leaf litter[25]. Dash and Patra[26] had reported higher levels of nitrogen in casts of *L. mauritii* than in the surrounding soil. This richness of nutrients in earthworm cast is a contribution of the gut resident bacteria. With the help of aerobic and anaerobic microflora earthworms transform waste into organic compost during vermicomposting[27]. Microbes in the cast of *L. mauritii* and *Eudrilus eugeniae* have been found to have phosphatase activity[11].

It is established that earthworms hold greater diversity of microbes in their gut, which are responsible for various activities, including mineralization and chelation of several ions in the soil[28]. Card *et al.*[29] showed that earthworm casts contain many more microbes than its surrounding soil because the intestines of earthworms inoculate the casts with microbes. Earthworms digest soil microbes selectively[30]. In a vermicomposting experiment by Arumugam *et al.*[31] with *L. mauritii*, the gut and casting analyses proved the removal of pathogenic *Salmonella*, *Shigella* and faecal coliform bacteria in 35 days, whereas *Pseudomonas*, cellulolytic *Bacillus* spp. and heterotrophic bacterial populations had increased at the end of the vermicomposting period, indicating the selective nature of earthworms.

In the above backdrop, the present study was conducted to isolate and identify the predominant gut bacteria from *L. mauritii.* The objective was to apply the selected microbe as a composting agent.

L. mauritii Kinberg collected from garden soil at the campus of University of Calcutta, Ballygunge was used in the experiment.

Since the concentration of beneficial microbes increases in the posterior part of earthworm gut[32,33], gut content from the hindgut, always beyond segment number 60, was collected by a sterile bacteriological loop and streaked on plates of nutrient agar and MacConkey medium.

Colonies with different morphological appearances were observed and counted. The colony count technique had been routinely used, as it is easy to perform and can be adopted for the measurement of populations of any magnitude[34]. The mean number of colony forming units (cfu) of bacteria, collected from 40 earthworm samples was calculated. The predominant type of colony was identified, isolated and streaked separately to get pure colonies. The bacterium in question was selected for identification by Gram-staining, malachite green staining, motility test and biochemical tests. Phylogenetic analysis by 16S rRNA molecular technique was done in the laboratory of GeNei™, Bangalore.

Table 1. Account of bacterial colony isolated from hindgut of *Lampito mauritii*

Colony type	Mean no. of CFUs
Licheniform, moderate size, irregular margin, flat, pink	36.23
Round pinhead size, entire margin, pink	1.07
Mucoid, small, round, yellow	0.00
Round, small, flat, translucent, dull pink	7.23
Water drop-like, moderate, dull pink	32.61

A suspension was made with the selected microbe in sterile distilled water and mixed with sterilized soil so that 1 g of soil contained 10^5 bacteria. This was added as composting agent with the organic material containing waste vegetables in a ratio of 100 g/kg. The experiment was set in circular fibre bowls at room temperature covered with sterile cotton cloth.

Composting was allowed for 60 days to mature and at the end of this period, the samples were oven-dried and analysed for nutrient content (NPK). The standard test methods were followed, viz. Kjeldahl distillation method for nitrogen estimation, Olsen's method to measure available phosphorus and ammonium acetate extracting method to estimate available potassium[35].

The compost thus obtained was applied as fertilizer for okra (*Abelmoschus esculentus*) plants at the dose of 9 kg compost/10 sq. m of soil. Seven days before seed planting, the soil was prepared by mixing the compost in soil. Okra seeds were soaked overnight in distilled water and in each earthen pot (inner diameter of the top 22 cm) four seeds were planted equidistantly. Absolute control set was maintained with ordinary soil without the compost.

In the present study the bacterial colony having licheniform shape, moderate size, flat elevation, irregular margin and pink colour (on MacConkey medium) was the most predominant (Table 1).

Morphologically, the bacterium was rod-shaped *Bacillus* sp. It was Gram-positive, endospore-forming and motile. It was designated as Bacillus sp. #202.

Biochemical tests reveal that the bacterium could produce catalase. It could ferment glucose, but not sucrose, rhamnose or arabinose. It had no potential to reduce nitrate to nitrite.

Based on nucleotide homology (Figure 1) and phylogenetic analysis (Figure 2), the microbe (*Bacillus* sp. #202) was detected to be *Bacillus cereus* (GenBank accession number: EU855219). Nearest homolog species was found to *Bacillus thuringiensis* (accession no. AB363741). Information about other close homologs for the microbe can be found from the Alignment Viewer table (Table 2).

Phosphate and potassium contents of the compost were high, whereas nitrate nitrogen was similar to the control in which no microbe was added to the starting material (Table 3).

Okra plants under the action of the compost yielded 87.5% more fruits over the control plants. The weight and length of okra were significantly higher (Table 4).

Table 2. Alignment Viewer using combination of NCBI GenBank and RDP database (sample *Bacillus* sp. #202)

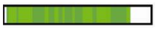
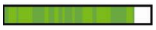
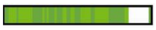
Alignment Viewer	ID	Alignment results	Sequence description
	*Bacillus* sp. #202	0.91	Studied sample
	EU855219	1.00	*Bacillus cereus* st CTSP45
	AB363741	1.00	*Bacillus thuringiensis* st NBRC 13866
	AF176321	1.00	*Bacillus anthracis*
	EU221418	0.98	*Bacillus mycoides* st L2S8
	DQ490406	0.99	*Bacillaceae bacterium* KVD-1971-02
	FM179766	0.97	*Acetobacter pasteurianus* st AUC13
	AM747227	0.97	*Bacillus pseudomycoides* st CIP 5259
	AM747230	0.99	*Bacillus weihenstephanensis* st WSBC 10204
	EU086578	0.92	*Bacterium* TLCL11
	AY345553	0.98	*Bacterium* H18

Table 3. Nutrient (NPK) value of the compost using *Bacillus* sp. #202 (*Bacillus cereus*)

Nutrient in compost	Value in control (kg/acre)	Value in compost (kg/acre)
Nitrate nitrogen (as N)	20.41	20.3
Available phosphate (as P_2O_5)	20.4	27.4
Available potassium (as K_2O)	45.36	52.1

CCGTGAATGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAA
GAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAAC
CTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAAC
ATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGG
ATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACAC
GGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGA
AAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA
CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACG
GTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGG
TTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCCGTGGAGGGTCATTGG
AAACTGGGAGACTTGAGTGCAGAAGAGAAAGTGGGAATTCCATGTGTACGGT
GAAATGCGTAAGGATATGGAGGAACACCAGTGGCCAAAGCGACTTTCTGCTC
TGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGC
CCTTTATTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGC
AAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGACCAT
GTGGTTTAATTCAAGCAACGAAATAACCTTACCAGGTCTTGACATCCTCTGAA
AACCCTACTATATACTGGCTTCTCCTTAGGAAGCAAAGAGACGGGTGGTGCA
TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCC
GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT
GACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACC
GCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGC
AACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGC
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTT
TGTAACACCCGAAGTCGGTGGGGTAACCTTTTGGAGCCAGCCGCCGTAAGGG
GGACCGAGGATCGGGAG

Figure 1. Aligned sequence data of sample *Bacillus* sp. #202 (1473 bp).

Table 4. Influence of compost on fruiting of okra

Fruiting result	Control plants	Plants with compost
Total number of fruits#	8	15
Mean length ± Std error	7.55 ± 1.45	9.85 ± 1.80*
Mean weight ± Std error	6.8 ± 1.81	11.64 ± 2.38**

#Total of five harvests. Std. error, Standard error. *P value of difference between control and test is significant at 0.10–0.05 level. **P value of difference between control and test is significant at 0.05–0.025 level.

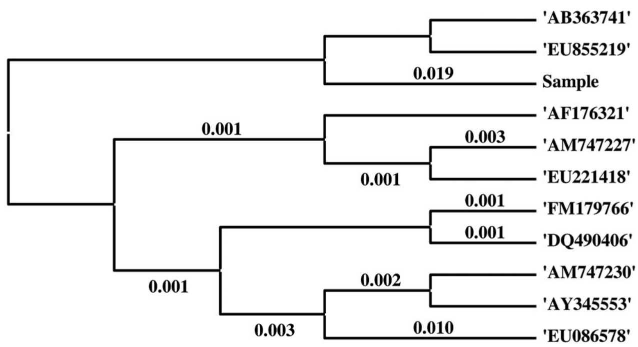


Figure 2. Phylogenetic tree (sample *Bacillus* sp. #202) made in MEGA 3.1 software using neighbour joining method.

The unique microconditions of the earthworm gut result in the selective stimulation of ingested soil microbes in such a way that desired and beneficial organisms are allowed to flourish therein[36]. According to Sruthy *et al.*[37], the bacteria in the foregut help to digest the food particles, actinomycetes in the midgut helps to destroy the pathogens by antagonistic activity, and the fungi help to bind the waste particles as castings in the hindgut. Uma Maheswari and Sudha[38] isolated phosphate solubilizing bacteria from the gut of earthworm varieties, among which *Bacillus subtilis* was the predominant one. In the present study, *Bacillus cereus* has been found as a predominant bacterium in *L. mauritii* gut. Various strains of *Bacillus* sp. are already found to be associated with composting. Muhammad and Amusa[39] found *B. cereus* and *B. subtilis* associated with cow dung, sawdust and rice husk composted soils. *B. cereus* in the present composting experiment increased phosphate and potassium content to a considerable extent. Composting by earthworm gut-resident bacteria has an advantage over vermicomposting, as the time taken for the process is less. Moreover, to process 1 tonne of organic matter daily through vermicomposting, it would require about 1500 sq. m of space with six workers[40]. This would produce about 70 tonne of earthworms casting annually[40]. Epigeic earthworms remain active throughout the year under favourable conditions. There is a constant monitoring of moisture levels, temperature and food for their survival and biomass production. Temperature below 35°C and moisture level between 40% and 60% are ideal for earthworm activity[41]. Therefore, this study opens up an avenue to clean the environment from organic wastes.

The compost obtained in the present work gave good results when applied in the soil as far as the number of fruits and their growth are concerned. Researchers found effective role of the microbe in plant growth. Jetiyanon *et al.*[42] found that the film coating of seeds with spores of *B. cereus* RS87 demonstrated early plant growth enhancement and indoleacetic acid was released from strain RS87 that would be one of the mechanisms for such a result. There remains immense possibility of inspecting the gut of other earthworms to find the dominant microflora with a view to explore their role in soil fertility enhancement by transforming waste into wealth.

1. Bot, A. and Benites, J., The importance of soil organic matter: key to drought-resistant soil and sustained food production. FAO Soils Bulletin 80, FAO, Rome, 2005, pp. 1–77.
2. Jones, C. G., Lawton, J. H. and Shachak, M., Organisms as ecosystem engineers. *Oikos*, 1994, **69**, 373–386.
3. Anderson, J. M., Soil organisms as engineers: microsite modulation of macroscale processes. In *Linking Species and Ecosystems* (eds Im Joies, C. G. and Lawton, J. H.), Chapman and Hall, London, 1995, pp. 94–106.
4. Kale, R. D. and Karmegam, N., The role of earthworms in tropics with emphasis on Indian ecosystems. *Appl. Environ. Soil Sci.*, 2010, 1–16; article Id 414356.
5. Lavelle, P., Earthworm activities and the soil system. *Biol. Fertil. Soils*, 1988, **6**, 237–251.
6. Lavelle, P., Functional domains in soils. *Ecol. Res.*, 2002, **17**, 441–450.
7. Shipitalo, M. J. and Protz, R., Chemistry and micromorphology of aggregation in earthworm casts. *Geoderma*, 1989, **45**, 357–374.
8. Barois, I., Villemin, G., Lavelle, P. and Toutain, F., Transformation of the soil structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. *Geoderma*, 1993, **56**, 57–66.
9. Shipitalo, M. J. and Protz, R., Factors influencing the dispersibility of clay in worm casts. *Soil Sci. Soc. Am. J.*, 1988, **52**, 764–769.
10. Sabrina, D. T., Hanafi, M. M., Nor Azwady, A. A. and Mahmud, T. M. M., Earthworm populations and cast properties in the soils of oil palm plantations. *Malays. J. Soil Sci.*, 2009, **13**, 29–42.
11. Vinotha, S. P., Parthasarathi, K. and Ranganathan, L. S., Enhanced phosphatase activity in earthworm casts is more of microbial origin. *Curr. Sci.*, 2000, **79**(9), 1158–1162.
12. Alauzet, N., Roussos, S., Garreau, H. and Vert, M., Microflora dynamics in earthworms casts in an artificial soil (biosynthesol) containing lactic acid oligomers. *Braz. Arch. Biol. Technol.*, 2001, **44**(2), 113–119.
13. Jayakumar, M., Karthikeyan, V. and Karmegam, N., Comparative studies on physicochemical, microbiological and enzymatic activities of vermicasts of the earthworms, *Eudrilus eugeniae*, *Lampito mauritii* and *Perionyx ceylanensis* cultured in press mud. *Int. J. Appl. Agric. Res.*, 2009, **4**, 75–85.
14. Bhatnagar, T., Earthworms and humification: a new aspect of microbial nitrogen incorporation induced by earthworms. In *Biodegradation and Humification* (eds Kilbertus, G. *et al.*), Pierron, Sarreguemines, France, 1975, pp. 169–182.
15. Teotia, S. P., Duley, F. L. and McCalla, T., Effect of stubble mulching on number and activity of earthworms. *Neb. Agric. Exp. Stn Res. Bull.*, 1950, **165**, 20.
16. Daniel, O. and Anderson, J. M., Microbial biomass and activity in contrasting soil material after passage through the gut of earthworm *Lumbricus rubellus* Hoffmeister. *Soil Biol. Biochem.*, 1992, **24**, 465–470.
17. Coleman, D. C., Crossley Jr, D. A. and Hendrix, P. F., *Fundamentals of Soil Ecology*, Elsevier Academic Press, Amsterdam, 2004, pp. 169–186.
18. Julka, J. M., Paliwal, R. and Kathireswari, P., Biodiversity of Indian earthworms – an overview. In Proceedings of Indo-US Workshop on Vermitechnology in Human Welfare, Rohini Achagam, Coimbatore, 2009.
19. Dash, M. C. and Patra, V. C., Density, biomass and energy budget of a tropical earthworm population from a grassland site in Orissa, India. *Rev. Ecol. Biol. Sol.*, 1977, **14**, 461–471.
20. Kale, R. D. and Krishnamoorthy, R. V., Cyclic fluctuations and distribution of three species of tropical earthworms in a farmyard garden in Bangalore. *Rev. Ecol. Biol. Sol.*, 1982, **19**, 67–71.
21. Reddy, M. V. *et al.*, Earthworm biomass response to soil management in semi-arid tropical Alfisol agroecosystems. *Biol. Fertil. Soils*, 1995, **19**(4), 317–321.
22. Tripathi, G. and Bhardwaj, P., Seasonal changes in population of some selected earthworm species and soil nutrients in cultivated agroecosystem. *J. Environ. Biol.*, 2004, **25**(2), 221–226.
23. Karmegam, N. and Daniel, T., Effect of physico-chemical parameters on earthworm abundance: a quantitative approach. *J. Appl. Sci. Res.*, 2007, **3**, 1369–1376.
24. Sathianarayanan, A. and Khan, A. B., Diversity, distribution and abundance of earthworms in Pondicherry region. *Trop. Ecol.*, 2006, **47**(1), 139–144.
25. Kale, R. D. and Krishnamoorthy, R. V., Litter preferences in the earthworm *Lampito mauritii*. *Proc. Indian Acad. Sci.*, 1981, **90**, 123–128.
26. Dash, M. C. and Patra, V. C., Density, biomass and energy budget of a tropical earthworm population from a grassland site in Orissa, India. *Rev. Ecol. Biol. Sol.*, 1977, **14**, 461–471.
27. Maboeta, M. S. and Van Rensburg, L., Vermicomposting of industrially produced woodchips and sewage sludge utilizing *Eisenia fetida*. *Ecotoxicol. Environ. Saf.*, 2003, **56**, 265–270.
28. Adnan, M. and Joshi, N., The uniqueness of microbial diversity from the gut of earthworm and its importance. *J. Microbiol. Biotech. Res.*, 2013, **3**(1), 111–115; http://scholarsresearchlibrary.com/archive.html
29. Card, A. B., Anderson, J. V. and Davis, J. G., Vermicomposting horse manure, Colorado State University Cooperative Extension No. 1.224, 2004, pp. 1–224; www.ext.colostate.edu/pubs/livestk/01224.html
30. Khomyakov, N. V., Kharin, S. A., Nechitailo, T. Y., Golyshin, P. N., Kurakov, A. V., Byzov, B. A. and Zvyagintsev, D. G., Reaction of microorganisms to the digestive fluid of earthworms. *Microbiology*, 2007, **76**, 45–54.
31. Arumugam, G. K., Ganesan, S., Kandasamy, R., Balasubramani, R. and Burusa, P. R., Municipal solid waste management through anaecic earthworm *Lampito mauritii* and their role in microbial modification. Green Pages, Eco Services International, August 2004; www.ecoweb.com

32. Parle, J. N., Activities of microorganisms in soil and influence of these on soil fauna. Ph D thesis, University of London, 1959.
33. Gomez-Brandon, M., Lazcano, C., Lores, M. and Dominguez, J., Detritivorous earthworms modify microbial community structure and accelerate plant residue decomposition. *Appl. Soil Ecol.*, 2010, **44**, 237–244.
34. Pelczar Jr, M. J., Chan, E. C. S. and Kreig, N. R., *Microbiology Concept and Application*, McGraw-Hill, New York, 1993, pp. 80–100; 158–161; 370.
35. Jackson, M. L., *Soil Chemical Analysis*, Prentice Hall of India Pvt Ltd, New Delhi, 1973, pp. 25–214.
36. Drake, H. L. and Horn, M. A., As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. *Annu. Rev. Microbiol.*, 2007, **61**, 169–189.
37. Sruthy, P. B., Anjana, J. C., Rathinamala, J. and Jayashree, S., Screening of earthworm (*Eudrilus eugeniae*) gut as a transient microbial habitat. *Adv. Zool. Bot.*, 2013, **1**(3), 53–56; http://www.hrpub.org
38. Uma Maheswari, N. and Sudha, S., Enumeration and detection of phosphate solubilizing bacteria from the gut of earthworm varieties. *J. Chem. Pharm. Res.*, 2013, **5**(4), 264–267; www.jocpr.com
39. Muhammad, S. and Amusa, N. A., *In-vitro* inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes. *Afr. J. Biotechnol.*, 2003, **2**(6), 161–164.
40. Gupta, P. K., Why vermicomposting? In *Vermicomposting for Sustainable Agriculture*, Agrobios (India), Agro House, Jodhpur, 2003, pp. 14–25.
41. Rajendran, P., Jayakumar, E., Kandula, S. and Gunasekaran, P., Vermiculture and vermitechnology for organic farming and rural economic development. Green pages, 2008; http://www.eco-web.com/editorial/080211.html
42. Jetiyanon, K., Wittaya-Areekul, S. and Plianbangchang, P., Film coating of seeds with *Bacillus cereus* strain RS87 spores for early plant growth enhancement. *Can. J. Microbiol.*, 2008, **54**(10), 861–867.

Received 23 January 2014; revised accepted 30 May 2014

Primitive breeding in an ancient Indian frog genus *Indirana*

Nikhil Gaitonde[1,]* and Varad Giri[2]

[1]National Centre for Biological Sciences, GKVK Campus, Bengaluru 560 065, India

[2]Western Ghats Regional Station, Bombay Natural History Society, Shrimati Niwas, B510, Patil Colony, Kolhapur 416 005, India

The Western Ghats biodiversity hotspot is rich in herpetofauna and harbours numerous endemic species. Unfortunately, many of these understudied species are threatened due to habitat loss, pollution, infectious diseases and climate change. *Indirana* (family Ranixalidae) is an ancient frog genus, endemic to the Western Ghats of India. Unlike most amphibians, it lays terrestrial eggs and has semi-terrestrial tadpoles. We barely have any knowledge about their development, life history, mating systems and reproductive ecology. Such information is crucial to design and implement successful conservation programmes. Hence, we studied the courtship, spawning behaviour and reproductive mode of an *Indirana* sp. from Amboli Reserve Forest located in the northern Western Ghats, Maharashtra, India. This species showcases a primitive type of inguinal amplexus and exhibits pronounced sexual size dimorphism, where females are significantly larger than the males. Average clutch size was 226 ± 41.5 eggs, with an egg diameter of 3.25 ± 0.32 mm. Fertilization rate was 87% with 100% hatching success. Additionally, this frog has evolved terrestrial eggs without the dependent traits like parental care and large egg size/small clutch size witnessed in other terrestrially egg-laying anurans (frogs and toads). This frog has reproductive mode 19, with its characteristic semi-terrestrial tadpoles. This genus represents the extreme of the trend (from obligatory aquatic to completely terrestrial) that amphibians show towards terrestriality.

Keywords: Amplexus, anuran, *Indirana*, reproductive mode, terrestrial eggs.

AMPHIBIANS exhibit a great diversity of reproductive modes, more than any other vertebrate group[1]. Diversification in reproductive modes reflects the environmental challenges that various species have overcome to successfully propagate a succeeding generation[1,2]. Behaviours associated with reproduction are usually species-specific and the defined set of courtship rituals usually concludes in the union of the gametes. In anurans (frogs and toads), fertilization being external, numerous ways such as peculiar sexual embrace and size assortative mating have evolved to achieve high fertilization success[3–5]. Anurans have various ways in which the male clasps the female during mating and spawning. This sexual embrace also known as 'amplexus' has evolved to juxtapose the male and female cloacae facilitating fertilization[3]. In primitive frogs like the Archeobatrachians, Myobatrachians, some Leptodactylids and Sooglosids, the amplexus is inguinal, where the male holds the female at the waist, anterior to her hind limbs. This type of amplexus is not as efficient as the axillary amplexus seen in Neobatrachians, where the male clasps the female near the arm pits and their cloacae are juxtaposed synchronizing semen ejaculation with oviposition, thus ensuring a high rate of fertilization[1,3]. Depending on the species, relative body size of the sexes, parental care and mode of oviposition, modifications of inguinal and axillary amplexus exist among species and are known as the cephalic, the straddle, the glued or the independent type[1].

Reproductive mode on the other hand is a combination of oviposition and developmental factors such as oviposition site, ovum and clutch characters and type of parental care[1–3]. Any particular type of reproductive mode seems to have evolved according to abiotic factors like temperature, precipitation, altitude and biotic factors such as predators or parasites[2,6]. Reproductive mode is also an integral part of the reproductive strategy employed by the species or the individual[3]. A large part

*For correspondence. (e-mail: nikhilng@ncbs.res.in)