

SHORT COMMUNICATION

Co-existence of ants and an arboreal earthworm in a myrmecophyte of the Indian Western Ghats: anti-predation effect of the earthworm mucus

Laurence Gaume¹, Megha Shenoy, Merry Zacharias and Renee M. Borges

Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India
(Accepted 15 October 2005)

Key Words: anti-predation mechanism, ant-plant, domatia, *Humboldtia*, India, *Perionyx*, repellent secretion, tropical forest

Arboreal earthworms are scarce in tropical rain forests, being confined to tree trunks or epiphytes (Fragoso & Rojas-Fernandez 1996). Their above-ground presence might be attributed to (1) temporary responses to flooding (Adis & Righi 1989), (2) permanent responses to acidity, flooding and anoxia of very wet forest soils (Lee 1983), (3) drought (Fragoso & Rojas-Fernandez 1996), (4) accidental events, e.g. mudslides (Picado 1911), or (5) anti-predation responses, e.g. escape from army ants (Gotwald 1995).

Because ants can be earthworm predators (Dejean *et al.* 1999), they are not expected to be involved in symbioses with them, with only two known exceptions (Laakso & Setälä 1997, Lenko 1972). Here, we report the co-occurrence of ants and the earthworm *Perionyx pullus* Stephenson (Oligochaeta, Megascolecidae), within the domatia of the myrmecophyte *Humboldtia brunonis* Wall. (Fabaceae, Caesalpinioideae). Studies on the invertebrate diversity of the ant-plants *Humboldtia laurifolia* Vahl (Krombein *et al.* 1999) in Sri Lanka and *H. brunonis* in India (Rickson *et al.* 2003) reported the occurrence of an oligochaete of the genus *Perionyx*, but did not mention an earthworm-ant co-existence.

Our findings raise three questions: (1) Is the earthworm *Perionyx pullus* arboreal? (2) Is it a permanent or temporary associate of the ant-plant? (3) Why is it not preyed upon by ants?

Humboldtia brunonis is an endemic myrmecophytic understory tree of the Indian Western Ghats whose domatia are occupied by a large diversity of microfauna, including numerous ants (Gaume *et al.* 2005a, b; Rickson

et al. 2003, Shenoy 2003). The ants feed at extrafloral nectaries of leaves, stipules and flower bracts. The earthworm genus *Perionyx* comprising 53 species, is endemic to the Oriental region (Julka & Paliwal 1993). Only juvenile earthworms were found in *H. brunonis* domatia; those reared in Bangalore reached maturity (developed a clitellum) in May–June. *Perionyx pullus* has a reduced gizzard making the worms unable to process soil.

During the dry season in March–April 1999 at Makut Reserve Forest, Coorg, Karnataka, in the southernmost *H. brunonis* range (altitude: 190 m, 12°05' N, 75°44' E, rainfall 5000 mm), we recorded *P. pullus* in 30 of 159 examined trees (18.9%, tree height = 5.6 ± 3.2 m) and 37 of 347 dissected domatia (10.7%). It was the third most abundant occupant after ants (62.8% of the domatia, ten species) and the centipede *Paracryptops indicus* Sylvestri (Cryptopidae) (13.2% of domatia). The occupancy for other taxa was: beetles (Carabidae): 5.2%, bees (*Braunsapis* sp., Apidae): 4%, roaches (Blattidae): 2.3%, and wasps (Sphecidae): 1.7%. We found worms only inside the domatia (up to seven per domatium, Figure 1a), while during the monsoon (June to October) we found no worms inside the 200 domatia examined, but observed a few, some clitellate, on *H. brunonis* leaves. Seventeen of the 37 earthworm-containing domatia also contained ants (*Technomyrmex albipes* Smith, *Crematogaster dohrni* Mayr, *C. wroughtonii* Forel and *Polyrhachis exercita* Walker) with inactive earthworms confined to one side, sometimes with a carton partition built by ants. Twelve of the 37 domatia contained earthworms alone, while eight contained other invertebrates also (sphecid wasps, roaches and the centipede *Paracryptops indicus*).

Domatium contents were also examined in the northernmost *H. brunonis* range (Agumbe Reserve Forest, Shimoga, Karnataka, altitude: 633 m, 13°50' N, 75°09' E, rainfall 7500 mm) in January 2004 during

¹Corresponding author. Address: Botanique et bioinformatique de l'architecture des plantes, UMR CNRS 5120, CIRAD - TA 40/PS 2, Boulevard de la Lironde, F-34398 Montpellier, cedex 5, France. Email: lgaume@cirad.fr

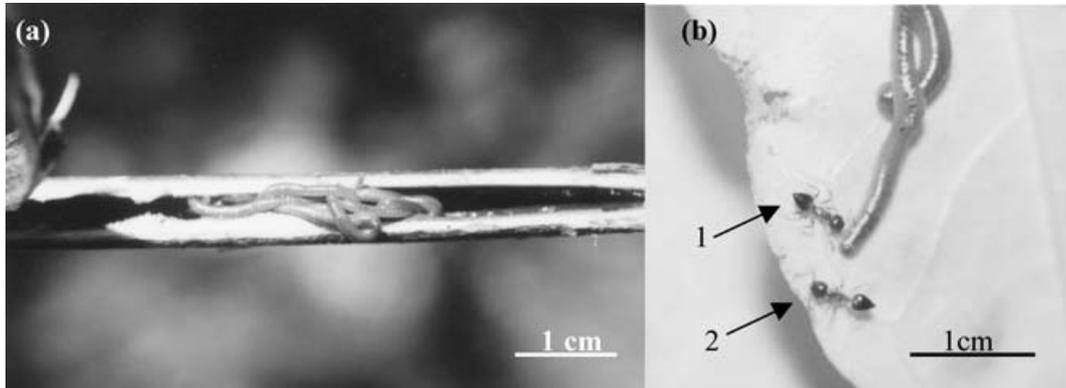


Figure 1. *Perionyx pullus*. (a) Juveniles within a caulinary domatium of *Humboldtia brunonis*. (b) Juvenile on a young leaf patrolled by workers of *Crematogaster wroughtonii*. After antennal contact with the earthworm (arrow 1), the workers exhibited a cleaning behaviour by rubbing their antennae against the young leaf (arrow 2).

the dry season. Juvenile earthworms were recorded in 24 of 52 examined trees (46.1%, significantly greater than in Makut, $\chi^2 = 15.3$, $P < 0.0001$) and in 76 of the 376 dissected domatia (20.2%, more than in Makut, $\chi^2 = 12.5$, $P < 0.0004$). However, only seven of the 76 earthworm-inhabited domatia also harboured ants (*Technomyrmex* sp., *C. dohrni*, *Polyrhachis gracilior* Forel and *Vombisidris humboldticola* Zacharias & Rajan) (less than in Makut, $\chi^2 = 20.1$, $P < 0.0001$). Ants at Agumbe (633 m) were less abundant than at Makut (190 m) since only 8.8% of the domatia were occupied by ants (against 62.8% in Makut; $\chi^2 = 115$, $P < 0.00001$). At Agumbe, 56 of the 76 domatia contained earthworms alone although earthworms also shared domatia (13 out of 76) with a great diversity of other arthropods (clerid beetles, forficulids, mirid and fulgoroid bugs, agelenid and salticid spiders, scolopendrid centipedes, polyxenid, spirobolid and polydesmid millipedes and springtails), some of which, e.g. centipedes, millipedes, spiders and beetle larvae, are potential ant predators. A brief study in January 2000 at a lower-elevation site in the same locality (Sitanadi, altitude: 333 m, 13°50' N, 75°09' E) also revealed a high frequency of earthworms and ants (of 19 domatia from three trees, five contained earthworms, of which four also contained *Crematogaster* or *Monomorium* ants). Nine ant species were thus found sharing domatia with *P. pullus* from the three sites pooled together.

Earthworm arboreality was investigated in Bisle Reserve Forest, Hasan District, Karnataka (altitude: 550 m, 12°40' N, 75°40' E) during dry (April 2002) and monsoon (September 2002) seasons. During the rains, we sampled around six trees whose domatia had been occupied by earthworms during the previous dry season and extracted earthworms from soil and leaf litter of four areas (each of 0.5 m²) around each tree following Gunn (1992). All accessible leaves and branches of all

six trees were thoroughly examined visually for 2 h for the presence of earthworms. During the rains, *P. pullus* was seen neither in soil nor in leaf litter nor in the domatia themselves, but juveniles were seen crawling on leaves in three of the six trees. Each stratum was occupied by only one species of earthworm (*P. pullus* on tree leaves, mean = 0.5 individuals, range = 0–1; *Wahoscolex* sp. (Octochaetidae) in the leaf litter in five of the six plots, mean = 2.8, range = 0–5; and *Pontoscolex corethrurus* Muller (Glossoscolecidae) in the soil of all six plots, mean = 5, range = 4–6).

At Makut, we investigated the mechanism underlying ant-earthworm co-existence. We studied the reaction to tethered earthworms of the three most common ant species found sharing domatia with earthworms. On each of 20 trees whose young leaves were patrolled by these ants (four trees by *Crematogaster dohrni*, six by *C. wroughtonii* and ten by *Technomyrmex albipes*), we counted the ants on one young leaf. We attached, using a small hook, a freshly collected earthworm, on the abaxial surface of one leaflet on each leaf (Figure 1b) and recorded ant behaviour for 10 min (Table 1). The second experiment focused on *T. albipes* alone because it is the most effective ant-patroller on *H. brunonis* (Gaume *et al.* 2005a, b). We studied ant reactions to earthworms compared to typical herbivores of *H. brunonis*, such as caterpillars, that we placed on *T. albipes*-patrolled young leaves. We compared the time of first ant contact, as well as the time of first ant-bite, for earthworms (10 young leaves of 10 different trees) and caterpillars (30 young leaves, 15 trees). The third experiment investigated ant reaction to earthworm mucus. On each of 10 trees patrolled by *Crematogaster* species, we counted ants feeding on foliar nectaries on one young leaf. We displaced the ants from the nectaries by gentle shaking and delicately rubbed earthworms on each nectary to apply mucus. We recorded ant behaviour, as well as numbers of ants feeding at

Table 1. Ant behaviour towards earthworms placed on *Humboldtia brunonis* leaves.

Ant	<i>Crematogaster dohrni</i> (n = 4)	<i>Crematogaster wroughtonii</i> (n = 6)	<i>Technomyrmex albipes</i> (n = 10)	Total (n = 20)
Ant number at t = 0 (mean ± SE)	5.2 ± 1.7	6 ± 1.8	14.4 ± 3.6	10.1 ± 2.1
Time to first contact (s) (mean ± SE)	45.7 ± 25.5	112 ± 32.7	24.5 ± 9.8	55.2 ± 14.3
Reversal (%)	100	100	90	95
Cleaning behaviour (%)	100	83.3	100	95
Zig-zag locomotion (%)	50	16.7	90	60
Time to first bite (s) (mean ± SE)	119 ± 28.9	390 ± 90	124 ± 37.5	156 ± 30.5
Ant number at 10 min (mean ± SE)	14.5 ± 13.9	3 ± 3.5	56 ± 24.5	31.8 ± 30.8
Predation at 10 min (%)	50	20	100	65

nectaries for 10 minutes. Control observations confirmed that, without mucus treatment, most of the ants returned to the nectaries in a few seconds after leaf disturbance.

In 95% of cases, ants moved away after first contact with the tethered earthworm, and usually cleaned their antennae (Table 1); ant locomotion also appeared altered with a zig-zag gait. Subsequent behaviour varied among ant species. Recruitment did not occur in *C. wroughtonii*, whose reaction after first contact was most frequently flight. *Technomyrmex albipes* exhibited predatory recruitment behaviour in all cases, *C. dohrni* only half the time. Consequently, the mean number of ants was higher on the leaf at the end of the experiment than at the onset (Wilcoxon signed-rank test: $Z = 3.2$, $n = 14$, $P = 0.001$). Ants usually found the earthworm in less than 1 min (Table 1). However, they only bit it 2.5 min after the onset of the experiment, once it had begun to dry. The time delay between the first antennation and the first bite reflected the repellent effect of fresh earthworms on ants. By comparison, the reaction of *T. albipes* to caterpillars was much more rapid and aggressive, with the time of first bite being significantly lower for caterpillars ($m = 56.3 \pm 15.2$ s, $n = 30$) than for earthworms ($m = 124 \pm 37.5$ s, $n = 10$, t-test, $t = -2.0$, $df = 38$, $P = 0.05$). Moreover, *T. albipes* consistently bit caterpillars as soon as they were found. Consequently, the interval between first contact by *T. albipes* and first bite was significantly lower for caterpillars (consistently zero, $n = 30$) than for earthworms ($m = 99.3 \pm 116$ s, $n = 10$, t-test, $t = -4.8$, $df = 38$, $P = 0.00002$). The behaviour of both *Crematogaster* species after mucus application on nectaries was unambiguous. All ants drinking at nectaries before the treatment, returned to the nectaries after the disturbance. However, after contact with the mucus, the ants retreated and exhibited antennal cleaning, especially when contact with the nectary took place soon after mucus treatment. During the first 7 min, none of the ants that contacted the nectaries, often visiting each one in turn, drank from them. At the end of the 10 min, only two ants drank at a nectary. Therefore, the number of ants drinking at nectaries at the end of the experiment ($m_{\text{end}} = 0.2$, range = 0–1) was significantly lower than

that at the onset ($m_{\text{onset}} = 7.3$, range = 3–14, Wilcoxon signed-rank test; $Z = 3.9$, $n = 10$, $P < 0.0001$).

The earthworm *Perionyx pullus* is thus shown to be arboreal and to co-exist symbiotically with ants and other invertebrates as a ubiquitous associate of the myrmecophyte *H. brunonis*. The presence of *P. pullus* in arboreal habitats during dry and wet seasons suggests that it is adapted to such environments and does not merely exploit them opportunistically. Juveniles mature inside the domatia during the dry season, and leave them to crawl on the tree leaves during the monsoon probably to mate. The genus *Perionyx* is more adapted to rainy environments, inhabiting mostly regions of high rainfall (Stephenson 1930). Our data therefore support the hypothesis of Fragoso & Rojas-Fernandez (1996) that arboreality, and confinement to domatia during the dry season, is a response to water shortage, as occurs in frogs which share the humid nest of ants (Rödel & Braun 1999).

The repellent effect of *Perionyx pullus* on contact against all ant species studied could explain their unexpected symbiosis with ants as well as other potential predatory arthropods. This effect is mucus-based since once desiccated, the earthworms were consumed by ants, and since ants clearly avoided mucus-treated nectaries, although nectaries are their principal food source. The active cleaning behaviour exhibited after earthworm contact suggests that ants attempted to remove mucus. Earthworm mucus probably causes avoidance of contact in earthworms sharing *Formica* ant nests (Laakso & Setälä 1997). Repellence might result from cytolytic and agglutinating properties of the coelomic fluid (Cooper & Roch 2003) and could also be directed against vertebrate predators, e.g. birds, that damage domatia in search of prey.

Could such an unusual ant-plant-earthworm association evolve towards a mutualism? The earthworm probably benefits from the anti-predation services of plant-ants deployed against invertebrates (Heil & McKey 2003). Reciprocally, it could favour the ant-plant protection mutualism as a third mediating partner (Gaume *et al.* 1998). Earthworms may process the pith within young domatia that ants need to remove and

could confer anti-fungal services to the ants or the host-tree, either because they are fungus-consumers (Laakso & Setälä 1997, Scheu 2003) or because their coelomic fluid provides protection against microbes (Cooper & Roch 2003). Earthworms might also provide nutrients to the tree by processing ant wastes. Carbon and nitrogen isotopic analyses have demonstrated the existence of nutrient exchanges inside domatia of some ant-plants (Sagers *et al.* 2000). Such exchanges are suspected to partly explain why domatia-bearing trees of *H. brunonis* produce more fruits than those without domatia (Gaume *et al.* 2005b). Further experiments are needed to assess costs and benefits of earthworms to plants and ants, as well as to clarify vital aspects of their biology.

ACKNOWLEDGEMENTS

The research was funded by a Romain Rolland post-doctoral fellowship (L. Gaume) from the French Ministry of Foreign Affairs, and by pre-doctoral support to M. Shenoy (R. M. Borges) from the Ministry of Environment and Forests, Government of India. We thank the Karnataka Forest Department for research permission, Erappan, Raghavan, Salaam, Suma Satish, C. Priyadarshini and Yettiraj for field assistance, T. M. Musthak Ali (University of Agricultural Sciences, Bangalore), B. Bolton (London Museum of Natural History) and J. M. Julka (Zoological Survey of India, Himachal Pradesh) for taxonomy of ants and earthworms. This manuscript was greatly improved by the comments of an anonymous reviewer, J. Blondel, D. McKey, J. Moog and P. Perret.

LITERATURE CITED

- ADIS, J. & RIGHI, G. 1989. Mass migration and life cycle adaptation – a survival strategy of terrestrial earthworms in Central Amazonian forests. *Amazoniana* 11:23–30.
- COOPER, E. L. & ROCH, P. 2003. Earthworm immunity: a model of immune competence. *Pedobiologia* 47:676–688.
- DEJEAN, A., SCHATZ, B., ORIVEL, J. & BEUGNON, G. 1999. Prey capture of *Psalidomyrmex procerus* (Formicidae: Ponerinae), a specialist predator of earthworms (Annelida). *Sociobiology* 34:545–554.
- FRAGOSO, C. & ROJAS-FERNANDEZ, P. 1996. Earthworms inhabiting bromeliads in Mexican tropical rainforests: ecological and historical determinants. *Journal of Tropical Ecology* 12:729–734.
- GAUME, L., MCKEY, D. & TERRIN, S. 1998. Ant-plant-homopteran mutualism: how the third partner affects the interaction between a plant-specialist ant and its myrmecophyte host. *Proceedings of the Royal Society of London, Series B* 265:569–575.
- GAUME, L., ZACHARIAS, M. & BORGES, R. M. 2005a. Ant-plant conflicts and a novel case of castration parasitism in a myrmecophyte. *Evolutionary Ecology Research* 7:435–452.
- GAUME, L., ZACHARIAS, M., GROSBOIS, V. & BORGES, R. M. 2005b. The fitness consequences of bearing domatia and having the right ant partner: experiments with protective and non-protective ants in a semi-myrmecophyte. *Oecologia* 145:76–86.
- GOTWALD, W. H. 1995. *Army ants: the biology of social predation*. Comstock Publishing Associates, Ithaca. 302 pp.
- GUNN, A. 1992. The use of mustard to estimate earthworm populations. *Pedobiologia* 36:65–67.
- HEIL, M. & MCKEY, D. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution and Systematics* 34:425–453.
- JULKA, J. M. & PALIWAL, R. 1993. A new species of *Perionyx* Perrier (Megascolecidae, Oligochaeta) from Northwest Himalaya, India. *Journal of the Bombay Natural History Society* 90:461–462.
- KROMBEIN, K. V., NORDEN, B. B., RICKSON, M. M. & RICKSON, F. R. 1999. Biodiversity of the domatia occupants (ants, wasps, bees, and others) of the Sri Lankan myrmecophyte *Humboldtia laurifolia* Vahl (Fabaceae). *Smithsonian Contributions to Zoology* 603:1–33.
- LAAKSO, J. & SETÄLÄ, H. 1997. Nest mounds of red wood ants (*Formica aquilonia*): hot spots for litter-dwelling earthworms. *Oecologia* 111:565–569.
- LEE, K. E. 1983. Earthworms of tropical regions – some aspects of their ecology and relationships with soils. Pp.179–194 in Satchell, J. (ed.). *Earthworm ecology*. Chapman & Hall, London.
- LENKO, K. 1972. Minhocas e sanguessugas (Annelida: Oligochaeta & Hirudinea) em ninhos de *Camponotus rufipes* (Insecta, Hymenoptera: Formicidae). *Revista Brasileira de Entomologia* 16:7–12.
- PICADO, M. C. 1911. Les Broméliacées épiphytes comme milieu biologique. *Comptes Rendus de l'Académie des Sciences de Belgique* 153:960–963.
- RICKSON, F. R., RICKSON, M. M., GHORPADE, K., NORDEN, B. B. & KROMBEIN, K. V. 2003. Invertebrate biodiversity (ants, bees and others) associated with stem domatia of the Indian myrmecophyte *Humboldtia brunonis* Wallich (Magnoliophyta: Fabaceae). *Proceedings of the Entomological Society of Washington* 105:73–79.
- RÖDEL, M.-O. & BRAUN, U. 1999. Associations between anurans and ants in a West African savanna (Anura: Microhylidae, Hyperoliidae, and Hymenoptera: Formicidae). *Biotropica* 31:178–183.
- SAGERS, C. L., GINGER, S. M. & EVANS, R. D. 2000. Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia* 123:582–586.
- SCHEU, S. 2003. Effect of earthworms on plant growth: patterns and perspectives. *Pedobiologia* 47:846–856.
- SHENOY, M. 2003. *An examination of a network of interactions. The myrmecophyte Humboldtia brunonis (Fabaceae) and its invertebrate associates*. Master's thesis, Indian Institute of Science, Bangalore, India.
- STEPHENSON, J. 1930. *The Oligochaeta*. Oxford University Press, Oxford. 978 pp.