Occurrence and distribution of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis indica* in Indonesia

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Abstract

Soil samples from 79 sites on five islands of Indonesia were baited with insects for the recovery of entomopathogenic nematodes. *Heterorhabditis* and *Steinernema* were equally prevalent, and were recovered from 11.7% of samples representing 20.3% of sites sampled. Both genera were recovered from coastal sites only. Entomopathogenic nematodes were more prevalent on the Moluccan islands of Ambon and Seram than on Java or Bali. They were not detected on Sulawesi, where non-coastal sites only were sampled. RFLP analysis was used in the identification of nematode isolates. *Heterorhabditis indica* was the only heterorhabditid identified. Two RFLP types of *Steinernema* were identified.

Introduction

Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) are promising biological control agents for a variety of insect pests. The freeliving stage of these nematodes, the infective juveniles (IJs), normally occur in soil from which they can be recovered by baiting with live insects (Bedding & Akhurst, 1975). Both genera have global distributions and numerous surveys have documented their occurrence in Australia (Akhurst & Bedding, 1986), Europe (e.g. Mráček, 1980; Vänninen *et al*., 1989; Hominick & Briscoe, 1990; Griffin *et al*., 1991) and the Americas (e.g. Mráček & Webster, 1993; Liu & Berry, 1995). The occurrence of entomopathogenic nematodes in tropical regions is less well documented, but surveys have been conducted in Hawaii (Hara *et al*., 1991), Sri Lanka (Amarasinghe *et al*., 1994) and peninsular Malaysia (Mason *et al*., 1996), and a recent survey of Japan included the subtropical Nansei-Shoto islands (Yoshida *et al*., 1998).

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The primary aim of the survey reported here was the isolation of indigenous strains of entomopathogenic nematodes for use in pest control projects in Indonesia (Fallon, 1998). The use of indigenous strains of natural enemy for pest control is preferred, both because of environmental considerations and because they are likely to be adapted to local climatic conditions. Entomopathogenic nematodes have not previously been isolated in Indonesia. The sampling programme reported here included five islands of the archipelago, including islands in both the Oriental and Australasian regions.

Materials and methods

The sampling strategy adopted was to collect soil from areas which were considered most likely to have populations of entomopathogenic nematodes, i.e. soils of coarse texture with good vegetation cover (Griffin *et al*., 1991, 1994a). Two sampling programmes were carried out during 1993, one in January–February (survey 1) in which soils were collected from both inland and coastal locations, and the second (survey 2) in July–August, when only coastal sites were sampled.

https://doi.org/10.1017/S0022149X00700812 Published online by Cambridge University Press

Survey 1

Sixty-four soil samples were collected from Central Java, West Java and South Sulawesi (fig. 1b,c) during January–February 1993 (wet season). More than one sample was taken from some sites. Forty three of the samples were from inland sites: from roadside verge (8), pasture (6), cultivation (22) and broadleaved (6) or coniferous (1) woodland. Twenty-one samples were taken close to the coast; these were predominantly taken in grassland, frequently with coconut palms present. Each sample consisted of approximately 40 pooled soil cores of 1.2 cm diameter, taken to a depth of about 10 cm. Cores were taken at intervals of a few paces by 'random walk' within a selected area.

The majority of samples (56) were transported to the laboratory at Maynooth and were baited with *Galleria mellonella* larvae (from laboratory culture) in screw capped glass jars of 300 ml capacity. There were five bait insects per jar, two jars per sample. Baited soils were incubated at 27°C. At intervals, the jars were inspected in the dark for bioluminescence. The presence of luminescent cadavers was used as an indication that the insects had been parasitized by *Heterorhabditis* spp., the bacterial symbiont (*Photorhabdus luminescens*) of which normally emits light. Luminescent cadavers were recovered from

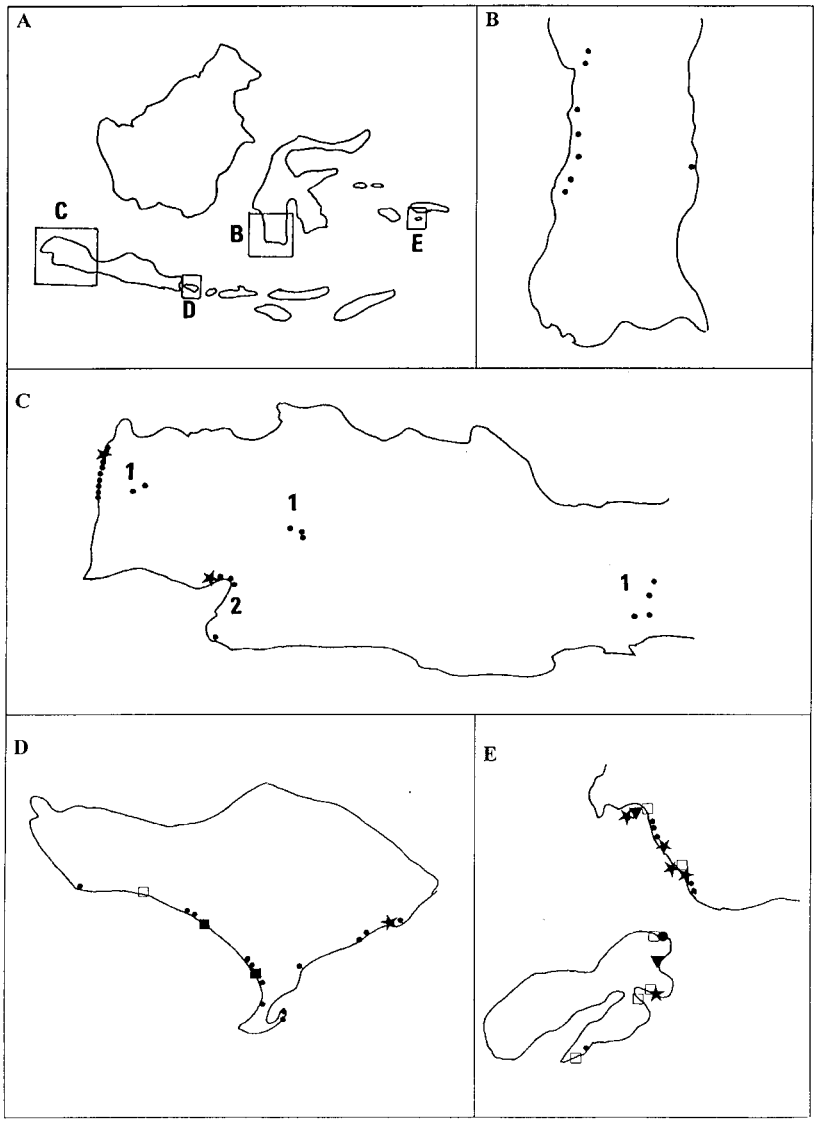

Fig. 1. Sites sampled for entomopathogenic nematodes in Indonesia. A: regions sampled (letters refer to other panels of fig.); B: South Sulawesi (survey 1); C: West and central Java (surveys 1 and 2); D, Bali (survey 2) and E: Ambon and west coast of Seram (survey 2). ★, *Heterorhabditis indica*; ●, unidentified *Heterorhabditis*; ■, *Steinernema* sp. SSL1 type; ▼, *Steinernema* sp. T87 type; □, unidentified *Steinernema*; •, site where entomopathogenic nematodes were not found.

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Table 1. Prevalence of entomopathogenic nematodes (EPN: *Heterorhabditis* and *Steinernema*) in Indonesia.

Region	Number of sites (and samples) Sampled	With *Heterorhabditis*	With *Steinernema*	Total with EPN	% of sites (samples) with EPN
Survey 1					
Central Java	4 (17)	0	0	0	0
W. Java	18 (34)	1 (1)	0	1 (1)	5.6 (2.9)
S. Sulawesi	8 (13)	0	0	0	0
Survey 2					
S. Java	10 (24)	1 (1)	0 (0)	1 (1)	10.0 (4.2)
Bali	21 (60)	1 (1)	4 (4)	5 (5)	23.8 (8.3)
Ambon	6 (40)	2 (3)	5 (6)	5 (9)	83.3 (22.5)
Seram	12 (35)	4 (7)	2 (4)	4 (10)	33.3 (28.6)

the soils as soon as they were detected; remaining insects were recovered after 7 days. Eight samples were processed at Sukamandi Research Institute, Java. These samples were baited with the larvae of an unidentifed lepidopteran, locally called 'bamboo insect', and cadavers were sent to Maynooth.

Survey 2

In July–August (dry season), samples were collected from the coastal fringe in three areas of Indonesia: West Java (south coast), Bali, and the Moluccan islands of Ambon and Seram (fig. 1c–e). At many of the sites chosen for sampling, the soil had a high sand content. Sampling was conducted under vegetation, including *Ipomoea* at the beach margins, grass, herbaceous plants and woodland. The method used was similar to that in survey 1, except that some of the samples were taken with a hand trowel. Both core and trowel samples each consisted of approximately 40 subsamples.

The samples were transported to the laboratory at

Table 2. Sites at which entomopathogenic nematodes (EPN) were isolated in Indonesia in two surveys conducted in January–February and July–August, 1993.

Location	Vegetation cover	EPN
W. Java		
near Anyer Kidul	Herbs/coconut	*H.i*
S. Java		
Cisolok	Woodland	*H.i*
Bali		
Candidasa	Grass/coconut	*H.i*
Surabratan	Ground cover including *Ipomoea*	*S* (SSL1)
Medawi	Grass/coconut	*S*
Basangkasa	Irrigated garden	*S* (SSL1)
Petingan	Grass/herbs	*S* (SSL1)
Ambon		
Latuhalat	Grass/coconut	*S*
Toisapu	Woodland	*S*
Natsepa	Grass/coconut	*S*, *H.i*
Waai	Grass/herbs/coconut	*S* (T87)
	Banana/herbs	*S* (T87)
Liang	Shrubs/coconut	*S*
	Herbs/coconut	*H*
	Woodland	*H*
Seram		
Kairatu ferry	Herbs/grasses	*H.i*
	Woodland	*H.i*
	Ground cover including *Ipomoea*	2*S*
E. of Kairatu	Shrubs/trees including coconut	*H.i*
Kamal	Scrub/trees	*S* (T87),
	Cassava	*H.i*
		**S* + *H.i*
Hatu Sua	Woodland	2 *H.i*

*Both *Heterorhabditis* and *Steinernema* in one sample.
H.i : *Heterorhabditis indica*; *H*: *Heterorhabditis* (not identified); *S* (SSL1): *Steinernema* SSL1 RFLP type; *S* (T87): *Steinernema* T87 RFLP type; *S*: *Steinernema* (not identified).

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Bogor for baiting. The baiting method was similar to that in survey 1, except that bait insects used were *Tenebrio molitor* (larvae and pupae), and 'bamboo insect'. Both insect species were obtained from Bogor market traders. Soil samples were moistened where necessary, and each was divided into two half-samples. One half-sample was baited initially with five *T. molitor* (three larvae and two pupae) while the other half-sample was baited with five bamboo insects. Baited samples were incubated at room temperature (28–32°C). They were checked visually after 2 days, both in light and in darkness; dead and/or glowing insects were removed. The remaining insects were recovered 4–5 (*T. molitor*) or 6 (bamboo insect) days after baiting, and each half-sample was baited again, using the other species of bait insect. Thus, each sample (as two half-samples) was baited with a total of 20 insects (ten *T. molitor* and ten bamboo insects).

Establishing nematode cultures

Insect cadavers were incubated on damp paper at 27°C (survey 1) or 28–30°C (survey 2) and transferred to modified White traps for emergence of IJs. Harvested IJs were tested for the ability to kill larvae of *G. mellonella* (survey 1) or *T. molitor* (survey 2), and cultures were established from the killed insects.

Molecular identifications

Initial identification to genus level was made on the basis of morphology. Identification of *Heterorhabditis* isolates to species level was by isoelectric focusing (IEF) (pH range 3–10) of soluble proteins as described by Joyce *et al*. (1994b). These identifications were confirmed by restriction enzyme digest of the internal transcribed spacer (ITS) region of the rDNA using the method of Joyce *et al*. (1994a). The amplified rDNA was digested with the *Hinf* I and *Alu* I restriction enzymes.

For *Steinernema*, PCR amplified products from the ITS region were digested with 17 different restriction enzymes and the RFLP profiles were compared with a database of profiles of *Steinernema* isolates (Reid *et al*., 1997). The Chinese isolate CB 2B of *S. longicaudum* Shen & Wang, 1992, which was included for comparison, was obtained from J. Heng, Institute of Biological Control, Beijing, China.

Results

Overall, entomopathogenic nematodes were recovered from 11.7% (26/223) of samples and 20.3% (16/79) of sites sampled in Indonesia in 1993. None of the samples from non-coastal locations (n = 43), and 14.4% (26/180) of samples from coastal locations were nematode-positive. The association of entomopathogenic nematodes with coastal location was significant (Chi-square = 5.70, P = 0.02).

Survey 1

A single entomopathogenic nematode isolate (identified as *Heterorhabditis indica* Poinar, Karunakar & David, 1992) was recovered from one (1.6%) of the 64 soil samples, representing 3.3% (1/30) of sites sampled in Java and Sulawesi in the wet season of 1993 (fig. 1, table 1). The positive sample was taken within 50 m of the sea on the west coast of Java, from a sandy soil under herbaceous cover and coconut palms (table 2). Failure to isolate entomopathogenic nematodes from inland locations, which constituted the majority of the samples in this survey, together with evidence of high prevalences of entomopathogenic nematodes in other coastal zones both in the tropics (Hara *et al*., 1991) and in temperate regions (Griffin *et al*., 1994a), led to a decision to concentrate further sampling on the coastal zone.

Survey 2

In this survey, all sites were within 1 km of the sea, and the majority were within 100 m of it. The recovery rate of entomopathogenic nematodes from samples from the four islands that were included in this survey differed significantly (Chi-square = 10.64, 3 d.f., P = 0.014). Less than 10% of samples from Bali and Java contained entomopathogenic nematodes, while nematodes were detected in 20–30% of samples from the Moluccan islands of Ambon and Seram (table 1). *Heterorhabditis* was recovered from all four islands, while *Steinernema* was not recovered on Java, but was isolated on Bali, Ambon and Seram (fig. 1; table 1). Both genera occurred together at each of four sites, and in one case (Kamal) both were isolated from the same soil sample (table 2). Thus, although nematodes were present in only 25 samples, 26 nematode isolates were recovered.

Table 3. Recovery of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema* from different habitats in Indonesia (survey 2, July–August, 1993).

Vegetation	Number of samples	No. (%) of samples with nematodes		
		Total nematodes	*Heterorhabditis*	*Steinernema*
Grass/herbs	25	3 (12.0)	1	2
Grass/herbs + coconut	48	7 (14.6)	3	4
Woodland/trees	54	10 (18.5)	7	3
Cultivation	5	*2 (40.0)	1	2
Ipomoea ground cover	16	3 (18.8)	0	3
Pandan	11	0	0	0

*Both *Heterorhabditis* and *Steinernema* in one sample.

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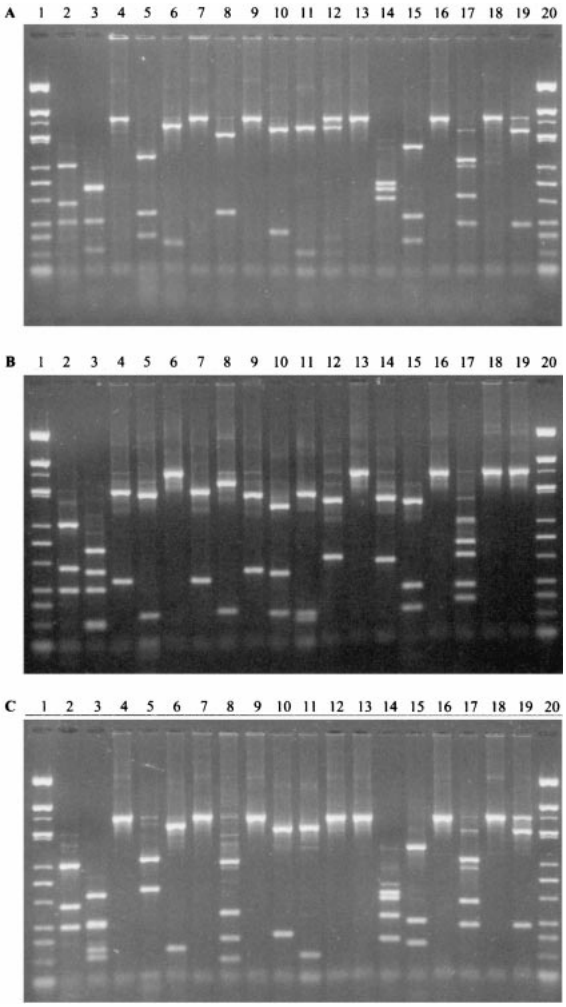

Fig. 2. Polymerase chain reaction amplification products from the internal transcribed spacer (ITS) region of rDNA digested with 17 different restriction enzymes. A, *Steinernema* sp. INA S15 (Ambon; T87 RFLP type); B, *Steinernema* sp. INA S9 (Bali; SSL1 RFLP type); C, *S. longicaudum* CB2B. For each gel, lanes 1 and 20 are molecular weight markers, band sizes 2000, 1200, 1000, 800, 750, 500, 400, 300, 200, 150, 100 and 50 base pairs, and lane 2 is a digest of *S. feltiae* (UK, site 76) with *Alu* I. Lanes 3–19 are individual digests of the respective isolate for that gel with the following restriction enzymes: 3, *Alu* I; 4, *Bst* 0; 5, *Dde* I; 6, *Eco*R I; 7, *Hae* III; 8, *Hha* I; 9, *Hin*d III; 10, *Hinf* I; 11, *Hpa* II; 12, *Kpn* I; 13, *Pst* I; 14, *Pvu* II; 15, *Rsa* I; 16, *Sal* I; 17, *Sau*3A I; 18, *Sau*96 I; 19, *Xba* I.

Table 4. Mean lengths (range) of infective juveniles of Indonesian strains of *Steinernema* sp. INA S3 (Bali; SSL1 RFLP type) and INA S22 (Seram; T87 RFLP type) and of *S. longicaudum*.

Species/strain	Length (μm) mean ± s.e (and range)	n
T87 RFLP type (INA S22)	958 ± 15.6 (800–1040)	20
SSL1 RFLP type (INAS3)	980 ± 21.6 (710–1120)	20
S. longicaudum (CB 2B)	1017 ± 22.3 (800–1200)	20

Nematodes were recovered from all of the habitats sampled, with the exception of 'pandan' (table 3). The soil associated with this vegetation type, which was dominated by *Pandanus* spp. and included other sclerophyllous vegetation, typically was very dry. There was no obvious association between habitat type and the presence of entomopathogenic nematodes, but sample sizes were too small for meaningful statistical analysis.

Most of the isolates were detected in the first baiting. The second baiting found entomopathogenic nematodes (all *Steinernema*) in four samples that were not shown positive by either of the insect species used in the first baiting. Both species of bait insect (*T. molitor* and bamboo insect) were successful in recovering entomopathogenic nematodes from soil samples. Eleven samples were identified as nematode-positive by *T. molitor* only (two *Heterorhabditis*, nine *Steinernema*), ten by bamboo insect only (six *Heterorhabditis*, four *Steinernema*), and five samples were identified by both insects (four *Heterorhabditis*, one *Steinernema*).

Identification

Of the nematodes isolated in both surveys, 52% (14/27) were *Steinernema* and the remainder (13/27) were *Heterorhabditis*. Not all of the isolates were identified to species level. All of the ten *Heterorhabditis* isolates that were identified were *H. indica*. Two RFLP types were detected amongst the 11 *Steinernema* isolates examined (table 2; fig. 2). Isolates from three sites on Bali were all of one RFLP type, with a profile identical to that of SSL1 (listed as Group 1 *Steinernema* from Sri Lanka by Amarasinghe *et al*., 1994). Three isolates from the Moluccas (two from Ambon and one from Seram), were of a different RFLP type, designated 'T87', which closely resembles the pattern of *Steinernema longicaudum* (fig. 2).

Measurements for one isolate of each RFLP type are given in table 4. Infective juveniles of the T87 and SSL1 RFLP types were similar in length and were shorter than IJs of *S. longicaudum* CB 2B (table 4).

Discussion

All of the nematodes, both steinernematids and heterorhabditids, were isolated from sandy coastal soils. An association of entomopathogenic nematodes with sandy coastal soils has also been demonstrated in surveys conducted in other regions of the tropics: Hawaii (Hara *et al*., 1991), Sri Lanka (Amarasingh *et al*., 1994) and Malaysia (Mason *et al*., 1996), and in subtropical Japan (Yoshida *et al*., 1998). In all five tropical/subtropical surveys including the present one, heterorhabditids were recovered only at the coast (within 4 km of the sea in subtropical Japan). A similar association has been noted for heterorhabditids in temperate regions (Griffin *et al*., 1994a, 1999; Liu & Berry, 1995). The reason for the association of certain entomopathogenic nematodes with coastal regions is not fully explained, but may relate either to the coastal location of sites or the sandy texture of soils, or both. Explanations involving proximity to the sea include those of Hara *et al*. (1991) who suggested that it might reflect recent introduction of the nematodes in Hawaiian soils. However, as evidence emerges that

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association of entomopathogenic nematodes with coastal soils is a widespread phenomenon, this explanation appears unlikely. Hominick *et al*. (1996) point to the occurrence of large populations of insects in washed-up marine detritus as providing suitable conditions under which entomopathogenic nematodes might flourish. This habitat could certainly provide a 'beach-head' for water-dispersed nematodes (Griffin *et al*., 1994b), but it is unlikely to account for the more general association of heterorhabditids with the coastal zone, as they are only rarely associated with unvegetated sand (Amarasinghe *et al*., 1994). While proximity to the sea *per se* cannot be discounted as a factor favouring the occurrence of heterorhabditids, the occurrence of extensive areas of very sandy soils at the coast must also be of importance. Both steinernematids and heterorhabditids, but especially the latter, are less likely to be found in soil with high clay content (Burman *et al*., 1986; Blackshaw, 1988; Vänninen *et al*., 1989; Griffin *et al*., 1991; Rueda *et al*., 1993; Stock, 1995), and in several instances where heterorhabditids have been found causing very high levels of natural parasitism, the soil was coarse textured (Sexton & Williams, 1981; Akhurst *et al*., 1992; Strong *et al*., 1996). Griffin *et al*. (1994a) suggest that extensive or contiguous areas of this soil type, such as are found at coasts, would provide opportunity for reintroduction from neighbouring sites following local extinction, if such metapopulation dynamics are important in the survival of members of this genus. It is likely that the extent of the preference of heterorhabditids for sandy soil varies between species. Species such as *H. bacteriophora* which occurs in silt loam (Campbell *et al*., 1995) may be less dependent on open textured soil than either *H. indica* (tropics) or the Irish type of *Heterorhabditis* (Griffin *et al*., 1994a).

While the association of *Heterorhabditis* with coastal sites is widespread and common, a similar association was noted for *Steinernema* in two of the tropical surveys. Steinernematids were found only at the coast in Indonesia (present survey) and Sri Lanka (Amarasinghe *et al*., 1994). However, they were isolated from both coastal and non-coastal sites in Malaysia (Mason *et al*., 1996) and only from inland sites in Hawaii (Hara *et al*., 1991). This may point to varying preferences among the local species of *Steinernema*. An association with the coast is not confined to tropical species of *Steinernema*: in Japan, the MY2 RFLP type *Steinernema* was isolated only from coastal sites in the warm temperate region (Yoshida *et al*., 1998).

There was no clear association of entomopathogenic nematodes with habitat type; both genera were recovered from a variety of vegetation types, both cultivated and uncultivated. Entomopathogenic nematodes were also isolated from a variety of vegetation types in Malaysia, and analysis did not show any association of steinernematids with crop type (Mason *et al*., 1996). Results from sub-tropical Japan provide further evidence that *H. indica*, at least, is not restricted by vegetation type: it was isolated from sites ranging from dry open land to wet, well-developed forest (Yoshida *et al*., 1998).

In survey 2, entomopathogenic nematodes were recovered at a higher rate from samples taken from coasts of the Moluccan islands of Ambon and Seram than from the south coasts of Java and Bali. While the wet season in Indonesia generally extends from October to April, in the Moluccas it is from April to July, and thus the time of this sampling fell at the end of the wet season in the Moluccas, but in the middle of the dry season elsewhere. However, samples taken on the west coast of Java during the wet season (survey 1) also resulted in low recovery of nematodes, suggesting that the low recovery on Java in the second survey was not due to a dry season effect. In support of this, Amarasinghe *et al*. (1994) repeatedly sampled 18 sites in Sri Lanka and found no relationship between the number of nematode-positive sites and season. Annual precipitation in the Moluccas is approximately double that on Java (Pearce & Smith, 1990) and this may favour entomopathogenic nematodes directly or through their hosts. Entomopathogenic nematodes were not detected on Sulawesi. However, as relatively few samples were taken there, and none was coastal, this cannot be taken as an indication of the status of entomopathogenic nematodes on the island.

Alfred Russel Wallace (1880) recognized a biogeographical discontinuity between the Indonesian islands of Bali and Lombok. Asian birds and mammals tended to occur to the west, Australian to the east. Whether by reference to George's (1981) revision of the Wallace line, or Michaux's (1994) partitioning of the Asian and Australian land masses, soils collected in our study from Java, Bali and Sulawasi came from the Asian side, while those collected in Ambon and Seram came from the Australian side. The data of table 2 show that while the tropically widespread *H. indica* was found on both sides of the Wallace line, *Steinernema* of the T87 RFLP type was recognized only from the Australian side, and *Steinernema* of the SSL1 RFLP type was recognized only from the Asian side. There are, in addition, a number of isolates of both *Steinernema* and *Heterorhabditis* from this survey that remain unidentified and these may show discontinuities associated with the Wallace line: persistent discontinuity in microbial populations across that line has been reported recently by Ploetz & Pegg (1997) who examined distribution of the microfungus causing *Fusarium* wilt of banana. However, the suggestion of a separation of *Steinernema* RFLP types across the Wallace line requires substantiation from additional sites.

The relationship of the *Steinernema* RFLP types to each other and to described species is unclear. The T87 RFLP profile is very similar to that of *S. longicaudum*, indicating that the two may be sister species or even conspecific. IJs of Indonesian isolates with the T87 profile were shorter than those of the Chinese isolate of *S. longicaudum* (table 4 and Stock *et al*., unpublished) but at 958 μm were similar in length to the Korean isolate of the species which measured 960 μm (Stock *et al*., unpublished). Steinernematids with similar profiles to each of the Indonesian RFLP profiles have previously been isolated in the Asian tropics: SSL 1 was one of three RFLP types of *Steinernema* isolated in Sri Lanka (Amarasinghe *et al*., 1994), and the T87 RFLP profile is very similar to that of one of the two steinernematid types recognized in peninsular Malaysia by Mason *et al*. (1996). *Steinernema longicaudum* has been recorded from both northern and southern hemispheres: originally isolated from Shandong province in China (Shen & Wang, 1992), it has also been found in Australia (Hominick *et al*., 1996), Korea and the USA (Stock *et al*., in press).

Galleria mellonella larvae were not available for baiting soils collected in survey 2. Had they been available, it is likely that higher prevalence of entomopathogenic nematodes would have been recorded. Rueda *et al*. (1993) demonstrated the superiority of *G. mellonella* compared to three other species of insect for the recovery of heterorhabditids and steinernematids from Tennessee nursery soils. However, each of the two insect species (*T. molitor* and bamboo insect) used in survey 2 was effective in recovering isolates of both *Heterorhabditis* and *Steinernema*.

When the results of our sampling programme in Indonesia (where up to 29% of samples or 83% of sites on an island yielded entomopathogenic nematodes) are considered together with those from Sri Lanka (25.6% of coastal sites positive (Amarasinghe *et al*., 1994)) and Malaysia (16.5% of coastal samples positive (Mason *et al.*, 1996)) it is clear that the coastal zone of the Asian tropics is a fruitful source of entomopathogenic nematodes. Thus, locally indigenous nematodes should be easily found for biocontrol programmes in the region.

Acknowledgements

This work was financially supported by the EU under the STD programme (TS3-CT92–0018). We are grateful to Yadi Suryadi, Yuliantoro Baliadi and Menaka Adnyana for assistance with sampling and to Suhendra and Rahmat for technical assistance. Dr Susan Joyce assisted with the identification of *Heterorhabditis*. We are also very grateful to Dr Patricia Stock and co-authors for access to their data prior to publication.

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(Accepted 22 September 1999)