

Speciation in the Acugutturidae (Nematoda: Aphelenchida)

Orville G. MARTI, JR ^{1,*}, Jean-François SILVAIN ² and Byron J. ADAMS ³

¹ United States Department of Agriculture, Agricultural Research Service, Crop Protection and Management Research Laboratory, P.O. Box 748, Georgia Coastal Plain Experiment Station, Tifton, GA 31793, USA

² Institut de Recherche pour le Développement, c/o CNRS, Laboratoire Populations, Génétique et Evolution, Bat. 13, B.P.1, 91198 Gif-sur-Yvette Cedex, France

³ University of Florida, Institute of Food and Agricultural Sciences, Entomology and Nematology Department, P.O. Box 110620, Gainesville, FL 32611, USA

Received: 25 March 2001; revised: 26 February 2002

Accepted for publication: 2 March 2002

Summary – Ectoparasitic nematodes (*Acugutturus*, *Vampyronema* and *Noctuidonema*) of the family Acugutturidae from 13 species of Lepidoptera (moths) and one species of Blattodea (cockroaches) were collected, measured and analysed by discriminant analysis in order to identify conspecifics and to determine which morphological characters were useful in defining differences between nematodes on different host species. The position of the excretory pore posterior to the metacarpus clearly differentiates *Acugutturus parasiticus* from *Noctuidonema* and *Vampyronema*. Two populations of *A. parasiticus* on *Periplaneta americana* from the West Indian islands of St Lucia and Guadeloupe are conspecific. Nematodes resembling *Vampyronema*, collected from two genera of Sphingidae in Guadeloupe are characterised by extremely long stylets, equal to ca 37% of the body length, and may represent a new genus. *Noctuidonema* from four species of *Spodoptera* (Noctuidae) are conspecific. However, a somewhat different *Noctuidonema* from the noctuid *Pseudaletia unipuncta* may represent a new species, based primarily on its shorter stylet length. Simple spicule structure, a smaller V percentage, and a shorter stylet in *Vampyronema* are characters that best define its differences from *Noctuidonema*. Nematodes on the noctuids *Anicla infecta* and *Eulepidotis addens* probably represent two new species of *Vampyronema*. There was no clear differentiation between nematodes on the noctuids *Mocis disseverans*, *M. latipes*, *Agrapha oxygramma* and *Orthodes crenulata*, and they are all probably conspecific with *V. dibolia*. Thus far, *Noctuidonema* has been found only on the trifine noctuids *Spodoptera* and *Pseudaletia*, whereas *Vampyronema* has been found on both trifine and quadrifine noctuids. We found evidence for sequential evolution, but not co-evolution, between these ectoparasitic nematodes and their lepidopteran hosts.

Keywords – *Acugutturus*, cockroaches, ectoparasitic nematodes, evolution, Lepidoptera, moths, *Noctuidonema*, *Vampyronema*.

The nematode family Acugutturidae was established by Hunt (1980) to accommodate a newly described species, *Acugutturus parasiticus* Hunt, an ectoparasite of the American cockroach, *Periplaneta americana* L., from the island of St Lucia in the West Indies. In establishing this genus and family, Hunt (1980) remarked that *A. parasiticus* appeared to have no close relatives among the Aphelenchoidoidea and was unique because of the great length of the stylet, 50-60 μm in adults. Other distinguishing features of *A. parasiticus* include the long length of the conus relative to the base and the absence of basal swellings or knobs on the stylet, the simple rose-thorn shaped spicules, the presence of a gubernaculum, and the position of the excretory pore posterior to the metacarpus (Hunt, 1980). In 1996, *A. parasiticus* was

found on *P. americana* from the West Indian island of Guadeloupe and on additional specimens collected from St Lucia (Marti *et al.*, 2000). However, none was found on cockroaches collected from mainland sites in French Guiana and Mexico.

In 1988, another ectoparasitic nematode, *Noctuidonema guyanense* Remillet & Silvain, was described on the noctuid moths *Spodoptera androgea*, *S. frugiperda*, *S. latifascia*, *S. marima*, *Anicla infecta* and on several species of *Leucania*, from French Guiana (Remillet & Silvain, 1988). The total length of the stylet reported for this species, 135-165 μm , was much longer than that of *A. parasiticus*, and the authors also mentioned the presence of basal knobs and an excretory pore located near the head. Anderson and Laumond (1990), while erroneously

* Corresponding author, e-mail: omarti@tifton.cpes.peadnet.edu

stating that the stylet of *N. guyanense* lacks basal knobs, provided additional details of the spicules, redescribed the male, and emended the diagnosis of the genus *Noctuidonema*.

Anderson and Laumond (1992) also described *N. daptria*, later redesignated as *Vampyronema daptria* by Hunt (1993), from the noctuid moth *Lesmone porcia* in Guadeloupe. This species differs from *A. parasiticus* in the anterior location of the excretory pore and in the shape of the spicules, which have ventral arms separated from blades and distinct elongated rostra. The spicules of *V. daptria* are simpler in form than those of *N. guyanense* and taper to fine points, in contrast to the bluntly pointed spicules of *Vampyronema dibolia* from *Mocis latipes* (Marti & Rogers, 1995). The stylet, however, is only 59–69 μm in females and is therefore much shorter than that of *N. guyanense*, but differs from that of *A. parasiticus* in having distinct knobs. *Vampyronema daptria* was not included in the present study because we collected no host specimens and no nematodes were available for measurement. Information about *V. daptria* in this report was based on Anderson and Laumond (1992).

Hunt (1993) revised the taxonomy of the Acugutturidae and established a new subfamily, the Noctuidonematinae, to include only the acugutturid ectoparasites of moths. *Acugutturus* was retained in the Acugutturidae, subfamily Acugutturinae, as a monotypic genus. Morphological characters distinguishing these subfamilies were primarily the small size and rose-thorn shape of the spicules of *Acugutturus*, the larger, more complex spicules present in *Noctuidonema*, and the simpler spicules in the newly created genus, *Vampyronema*, which exhibited characters sufficiently different from *Noctuidonema* to justify separate generic status.

The only other species of Acugutturidae described to date is *V. dibolia* (Marti & Rogers), from the noctuid moth *Mocis latipes* in south Georgia, USA (Marti & Rogers, 1995). This species has a stylet length of 83–114 μm in females, an excretory pore located near the head, and simple spicules somewhat different in size and shape from those of *V. daptria*. In addition, the distribution of *V. dibolia* over the body of its host is distinctly different from that of *N. guyanense*. On *M. latipes*, a large portion of the nematodes may frequently be found on the anterior abdomen, whereas on *S. frugiperda*, the nematodes are found primarily on the posterior abdomen (Rogers & Marti, 1992, 1993, 1994). In other species of *Mocis* (*M. disseverans*, *M. texana* and *M. marcida*), the nematodes were distributed in a manner similar to those

on *M. latipes*. Similarly, in other species of *Spodoptera* (e.g., *S. dolichos*, *S. latifascia* and *S. ornithogalli*), the nematode distribution was similar to that found on *S. frugiperda* (Marti, unpubl.). This difference in distribution on different host genera is quite striking and appears to be a distinct biological difference between *Noctuidonema* and *Vampyronema*.

Acugutturid nematodes insert their stylet through the cuticle of the host and ingest haemolymph (Hunt, 1980; Marti *et al.*, 1990). Infestations of *N. guyanense* on the noctuid *S. frugiperda* also reduce longevity and egg fertility (Simmons & Rogers, 1994; Marti & Rogers, 2000). These studies indicate that the nematodes are parasitic and not merely phoretic or commensal.

Since 1987, ectoparasitic nematodes have been collected from a variety of Lepidoptera from Fiji, French Guiana, Guadeloupe and the southeastern United States of America (Rogers *et al.*, 1990a, b, 1997; Marti *et al.*, 2000). Many of these nematode specimens have been examined and measured. Although *Acugutturus* is easily defined by its specific suite of characters, there are few discrete characters defining differences between Noctuidonematinae parasitising the Lepidoptera. With the exception of obvious differences in spicule type between nematodes parasitising *Spodoptera* and *Mocis* spp., all morphological characters examined appeared to be continuously variable rather than discrete.

We used discriminant analysis to determine which characters, if any, were important in defining differences between nematodes from different host species. Of particular interest was the possibility of determining whether any of the nematodes from different host species represent independently evolving lineages (species) according to the view "... that a species not be a speculative statement, made solely on the basis of a definable 'difference', but rather predictive, based on evidence that suggests the two prospective species are on independent trajectories..." (Adams, 1998).

Materials and methods

Ectoparasitic nematodes measured in this study were obtained from 13 species of Lepidoptera and from two populations of *Periplaneta americana* (Blattodea) collected from several different localities (Table 1). *Periplaneta americana* from St Lucia were collected in 1997 by Jeanette Williams. All Lepidoptera and Blattodea specimens from Guadeloupe were collected in 1996 (Marti *et al.*, 2000). No male nematodes were found on *Xylo-*

phanes chiron. Lepidoptera specimens from Tifton, Georgia, USA, were collected between 1988 and 1997. A total of 15 groups of female nematodes and 14 groups of males were studied. Nematodes from three of the 15 host species have been previously described. These were *Acugutturus parasiticus* Hunt from *P. americana*, *Noctuidonema guyanense* Remillet & Silvain from *Spodoptera* spp. and several other noctuids, and *V. dibolia* (Marti & Rogers) from *Mocis latipes*. A fourth species, not included in the present analysis, was *V. daptria* (Anderson & Laumond).

Captured cockroaches were preserved whole in 10% buffered formalin. Lepidoptera were collected from Universal traps baited with species-specific pheromone lures, or at night from a white sheet illuminated by a 125 or 160W mercury vapour light powered by a portable electric generator. Abdomens were removed from moths and placed into labelled vials containing 10% buffered formalin. Selected nematode specimens were later processed through an alcohol series to glycerin and mounted permanently on glass slides. Specimens were studied by transmitted light, differential interference contrast, and phase contrast optics. Measurements of morphological characters were obtained with a Nikon Optiphot photomicroscope fitted with a calibrated ocular micrometer. All measurements are in μm . Characters based on ratios or percentages were calculated from appropriate original data.

Statistica, version 5.5 (StatSoft Inc., Tulsa, OK, USA) was used for all statistical analyses and graph production.

STATISTICAL PROCEDURE

A total of 27 characters was measured from each specimen of each gender, plus spicule and rostrum length in males and egg width and length in females. From these 29 characters per gender, an additional three percentage and six ratio characters were calculated from the original data, giving a total of 36 morphological variables in the final dataset. However, not all characters could always be found or adequately measured in all specimens. In the final dataset, therefore, 18 morphological characters in the males and 16 in the females were used, in addition to the six ratios and three percentages. The characters were as follows: maximum body width, stylet length, bulb length, bulb width, head width, head length, spicule length along the curve (males), gonad length, tail length, excretory pore to anterior end, anterior gonad to base of bulb, anterior end to base of bulb, body width at cloaca or vulva, anterior end to cloaca or vulva, body width at nerve ring, anterior end to anterior gonad, body length, rostrum length (males), a (body length/greatest body width), b (body width/distance from anterior end to base of bulb), c (body length/tail length), c' (tail length/body width at vulva and/or cloaca), V (cloaca or vulva to anterior end \times 100/body length), G (reproductive system \times 100/body length), gonad length as

Table 1. Host species, family, sub-family, collection locality, and numbers of ectoparasitic nematodes measured.

Host species	Code ¹⁾	Family	Subfamily	Locality	Nematodes
<i>Periplaneta americana</i> L.	pa	Blattidae ²⁾	Blattinae	St Lucia	5♂ 13♀
<i>Periplaneta americana</i> L.	pa2	Blattidae	Blattinae	Guadeloupe	7♂ 18♀
<i>Spodoptera dolichos</i> (Fabricius)	sd	Noctuidae ³⁾	Amphipyridae	GA, USA	10♂ 5♀
<i>Spodoptera frugiperda</i> (J.E. Smith)	sf	Noctuidae	Amphipyridae	GA, USA	11♂ 17♀
<i>Spodoptera latifascia</i> (Walker)	sl	Noctuidae	Amphipyridae	GA, USA	6♂ 12♀
<i>Spodoptera ornithogalli</i> (Guenée)	so	Noctuidae	Amphipyridae	GA, USA	10♂ 10♀
<i>Eulepidotis addens</i> (Walker)	ea	Noctuidae	Catocalinae	Guadeloupe	2♂ 15♀
<i>Mocis disseverans</i> (Walker)	md	Noctuidae	Catocalinae	GA, USA	4♂ 9♀
<i>Mocis latipes</i> Guenée	ml	Noctuidae	Catocalinae	GA, USA	16♂ 39♀
<i>Orthodes crenulata</i> (Butler)	oc	Noctuidae	Hadeninae	GA, USA	4♂ 9♀
<i>Pseudaletia unipuncta</i> (Haworth)	pu	Noctuidae	Hadeninae	GA, USA	9♂ 18♀
<i>Anicla infecta</i> (Ochsenheimer)	ai	Noctuidae	Noctuinae	GA, USA	12♂ 15♀
<i>Agrapha oxygramma</i> (Geyer)	ao	Noctuidae	Plusiinae	GA, USA	7♂ 13♀
<i>Perigonia lusca</i> Fabricius	pl	Sphingidae ³⁾	Macroglossinae	Guadeloupe	4♂ 19♀
<i>Xylophanes chiron</i> Drury	xc	Sphingidae	Macroglossinae	Guadeloupe	0♂ 18♀

¹⁾ These codes are used to identify host species in the figures.

²⁾ Blattodea (cockroaches). For enumeration purposes, *P. americana* from St Lucia and Guadeloupe were counted as separate hosts.

³⁾ Lepidoptera (moths).

% of body length, stylet length as % of body length, and excretory pore to anterior end as % of body length.

Male and female nematode untransformed data were analysed and plotted separately because different suites of characters are involved for each gender. Multivariate analysis (MANOVA) was used to determine whether there were differences between nematodes from different host groups based on the entire suite of characters for each nematode gender. Each character was then analysed with univariate analysis (ANOVA) to determine if the character contributed significantly to differentiating between at least two host groups for each nematode gender. The Bonferroni test was used to check against overestimation of significance of univariate characters. These procedures were described by Footit and Sorensen (1992) and Sanmartín and Martín-Piera (1999).

Forward stepwise discriminant analysis was used to identify which morphological characters made significant contributions to defining differences among nematodes from different host species. Non-significant and redundant characters were identified and deleted from the models. Four separate analyses were conducted for male and female nematodes. First, nematodes from all host species listed in Table 1 were included ($n = 15$ female and $n = 14$ male groups). Based on these results, nematodes on Blattodea and Sphingidae were deleted from the second step, which included only nematodes from noctuid hosts. Again, morphological characters were ranked and non-significant ($P > 0.05$) and redundant characters were identified and deleted from the model. In the third step, only nematodes from groups having simple spicules similar to those of *V. dibolia* were included ($n = 6$ female and $n = 6$ male groups). The same procedure was followed in the last step, which analysed only nematodes having complex spicules similar to those of *N. guyanense* ($n = 5$ female and $n = 5$ male groups). This procedure produced eight models, four for each nematode gender, for discriminating between nematodes from all 15 host groups, between nematodes from noctuid hosts, between nematodes having simple spicules (*Vampyronema*), and between nematodes having complex spicules (*Noctuidonema*).

Discriminant functions were used to calculate posterior probabilities that individual nematodes were classified correctly into their respective groups. Separate classifications were obtained for male and female nematodes. A high percentage of correct classification is a measure of the discriminatory power of the morphological characters (Footit & Sorensen, 1992). Discriminant functions were evaluated for significance with chi-square and only signif-

icant ($P < 0.05$) functions were used in data interpretations. Standard discriminant function coefficients, the correlations of the morphological characters to the discriminant functions, were used to determine which morphological characters contributed most to the differentiation between groups. A large absolute value of the standard discriminant function coefficient indicates that a character is strongly represented by the discriminant function.

Two-dimensional graphs were produced that provided the best differentiation between nematodes from different host species for each nematode gender. Clearly defined groups were marked by 95% ellipses, which delimit the area in which a new observation is likely to occur. Results from both nematode genders from each host species were evaluated to arrive at a consensus regarding the status of the nematodes from each host group.

The most important morphological characters identified by discriminant analysis were used in cluster analysis to produce tree diagrams for each nematode gender. Data for each morphological character were standardised with mean = 0 and standard deviation = 1. Clusters were based on Euclidean distance for the distance measure between groups and on complete linkage for the amalgamation rule. Tree diagrams were scaled to $(Dlink/Dmax) * 100$ and were then evaluated and compared to results from discriminant analysis to arrive at a consensus regarding similarities between nematodes from different host species.

EXAMINATION OF NEMATODES FROM ADDITIONAL HOST SPECIES

We also examined additional nematodes from other lepidopteran hosts from French Guiana and the Fiji Islands. These nematodes were not measured in detail because they were available only in small numbers, were of uneven quality, or both. In all of the specimens from Noctuidae, however, we were able to make a determination regarding their generic placement. We were able, therefore, to test our conclusions regarding the value of specific characters in defining the association of nematode clades with clades and sub-families of the Noctuidae.

Results

MANOVA showed differences, regardless of nematode gender, among nematodes from the 15 host species, based on all morphological characters. All F values were highly significant ($P < 0.001$, $df = 13, 91$ for males

and 14, 113 for females). In addition, ANOVA showed that each morphological character contributed to the differentiation of nematodes of each gender from at least two host species. All F values were highly significant ($P < 0.001$, $df = 11-13$, 83-93 for males and 14, 209-214 for females). Therefore, all 18 characters, six ratios, and three percentages listed above were used in subsequent discriminant analysis to determine which were most useful in distinguishing between nematodes from different hosts.

STEP 1: 15 HOST SPECIES (BLATTODEA AND LEPIDOPTERA)

Discriminant analysis of female nematodes from 15 host species revealed three separate groups, *i*) from the two *Periplaneta americana* populations (*Acugutturus parasiticus*); *ii*) from the two species of Sphingidae; *iii*) all other host species (Fig. 1A, B). There were ten significant canonical roots, accounting cumulatively for 99.78% of the explained variance between groups. The first two roots accounted for 90.78% of the explained variance. Examination of the factor structure matrix, the correlation of the morphological characters to the canonical roots, showed that the distance of the excretory pore from the anterior end, the stylet length, and V were the characters most important in distinguishing between female nematodes from the 15 host species (Table 2). The other characters were of less importance, although all were significant for female nematodes.

Discriminant analysis of male nematodes from 14 host species (no male nematodes were found on *Xylophanes chiron*) revealed four distinct groups (Fig. 1B; Table 2). Canonical roots 1 (excretory pore) and 3 (stylet length) gave the best differentiation among males. In addition to the differentiation of *A. parasiticus* and the male nematodes on *Perigonia lusca* from each other and from the remaining host groups, it was also found that male nematodes from *Pseudaletia unipuncta* were different from those collected from the four species of *Spodoptera*. The two populations of *A. parasiticus* did not differ, but were distinct from the other groups, primarily due to the difference in the position of the excretory pore.

In the males, there were eight significant ($P \leq 0.05$) canonical roots, accounting cumulatively for 99.44% of the explained variance among males. The first three canonical roots accounted for 94.46% of the explained variance. Canonical roots 1 and 3 are represented primarily by the excretory pore and the stylet length, respec-

tively, whereas root 2 (not shown) is represented primarily by spicule length.

STEP 2: 11 HOST GROUPS (NEMATODES OF LEPIDOPTERA: NOCTUIDAE)

Analysis of morphological characters of nematodes parasitising only noctuids provides additional information differentiating several of these groups. Among the females, there were ten significant canonical roots, accounting cumulatively for 100% of the explained variance. The first three roots accounted cumulatively for 80.6% of the explained variance, with V , stylet length and bulb width being the most important (Table 3). V was by far the most important female character differentiating nematodes among the noctuid hosts, and was more useful than tail length alone. V provides clear separation of female nematodes of *Pseudaletia* and *Spodoptera* from the other noctuid groups (Fig. 1C). The former group is characterised by $V > 92\%$ (*i.e.*, short female tails) and by complex spicules and the latter group by $V < 92\%$ (*i.e.*, longer female tails) and simple spicules.

Among male nematodes from noctuid hosts (Fig. 1D; Table 3) discriminant analysis provided clear differentiation in root 1 between males having complex spicules (nematodes from *Pseudaletia* and *Spodoptera*) *vs* those having simple spicules (nematodes from *Agrapha*, *Anicla*, *Eulepidotis*, *Mocis* spp., and *Orthodes*). There were nine significant canonical roots, which cumulatively accounted for 99.83% of the explained variance among groups. The first three roots, accounting cumulatively for 92.0% of the explained variance among groups, were represented primarily by spicule length, stylet length and stylet length as a percentage of body length, respectively.

Nematodes from *Pseudaletia* can be separated from those on *Spodoptera* by the shorter stylet length (Fig. 1C, D). Nematodes on *Agrapha oxygramma* can be separated from *M. latipes* primarily on the basis of the shorter stylet length ($P \leq 0.05$). Similarly, males on *Anicla infecta* have shorter spicules ($P \leq 0.05$) than the nematodes on the remaining host groups.

Only two male nematodes from *E. addens* were available. However, these were distinct from nematodes on *M. latipes*. The female nematodes on *E. addens* were also well differentiated from those on *M. latipes*, despite some overlap (Fig. 2A).

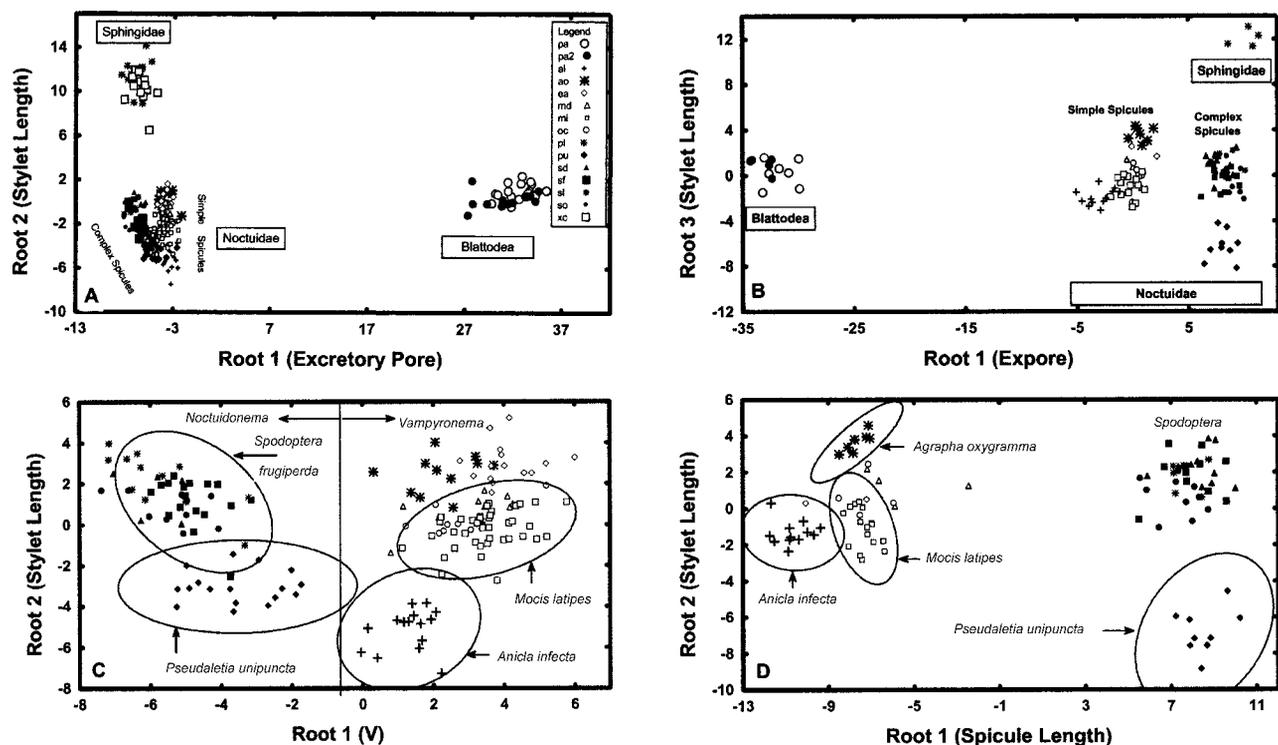


Fig. 1. Plot of discriminant functions for nematode morphometric characters. Ellipses indicate 95% confidence limits for selected host species. A: Female nematodes from 15 host species (Blattodea and Lepidoptera); B: Male nematodes from 14 host species (Blattodea and Lepidoptera); C: Female nematodes from 11 host species (Lepidoptera); D: Male nematodes from 11 host species (Lepidoptera). Legend (Host Names): pa, Periplaneta americana (St Lucia); pa2, P. americana (Guadeloupe); ao, Agrapha oxygramma; ai, Anicla infecta; ea, Eulepidotis addens; md, Mocis disseverans; ml, M. latipes; oc, Orthodes crenulata; pl, Perigonia lusca; pu, Pseudaletia unipuncta; sd, Spodoptera dolichos; sf, S. frugiperda; sl, S. latifascia; so, S. ornithogalli; xc, Xylophanes chiron.

Table 2. Summary of forward stepwise discriminant analysis for male and female nematode morphological characters from 14 and 15 host species, respectively.

Step	Males – all groups (n = 14)		Females – all groups (n = 15)	
	Character	F (df1, df2)	Character	F (df1, df2)
1	Excretory pore	403.13*** (13, 93)	Excretory pore	616.96*** (14, 214)
2	Spicule length	209.71*** (13, 92)	Stylet length	167.78*** (14, 213)
3	Stylet length	59.08*** (13, 91)	V	152.81*** (14, 212)
4	Vulva or cloaca to ant. end	11.55*** (13, 90)	Bulb width	17.56*** (14, 211)
5	Ant. end to post. bulb	9.05*** (13, 89)	Excretory pore, % of body	14.00*** (14, 210)
6	Stylet, percent of body	5.82*** (13, 88)	a	10.50*** (14, 209)
7	B	7.02*** (13, 87)	Ant. end to post. bulb	9.99*** (14, 208)
8	G	6.40*** (13, 86)	G	7.74*** (14, 207)
9	Bulb width	4.47*** (13, 85)	Vulva or cloaca to ant. end	10.23*** (14, 206)
10	Body width at nerve ring	4.19*** (13, 84)	b	8.26*** (14, 205)

***P ≤ 0.001.

Table 3. Summary of forward stepwise discriminant analysis for male and female nematode morphological characters from 11 species of host Noctuidae (Lepidoptera).

Males – Lepidoptera groups ¹⁾ (n = 11)				Females – Lepidoptera groups ¹⁾ (n = 11)		
Step	Character	F	(df1, df2)	Character	F	(df1, df2)
1	Spicule length	240.39***	(10, 80)	V	109.86***	(10, 150)
2	Stylet length	25.10***	(10, 79)	Stylet length	61.94***	(10, 149)
3	Stylet, % of body length	13.88***	(10, 78)	Bulb width	18.30***	(10, 148)
4	Cloaca to anterior end	9.81***	(10, 77)	Excretory pore, % of body	9.82***	(10, 147)
5	Rostrum length	7.62***	(10, 76)	Vulva to anterior end	9.35***	(10, 146)
6	Ant. end to ant. gonad	7.00***	(10, 75)	Body width at nerve ring	8.73***	(10, 145)
7	G	7.95***	(10, 74)	Stylet, % of body length	7.77***	(10, 144)
8	c'	6.51***	(10, 73)	Tail length	7.01***	(10, 143)
9	Body width at nerve ring	6.15***	(10, 72)	Head width	6.72***	(10, 142)
10	Bulb width	5.48***	(10, 71)	Gonad, % of body length	5.95***	(10, 141)

¹⁾ Not including *Perigonia lusca*, *Xylophanes chiron* or two populations from *Periplaneta Americana*.

***P ≤ 0.001.

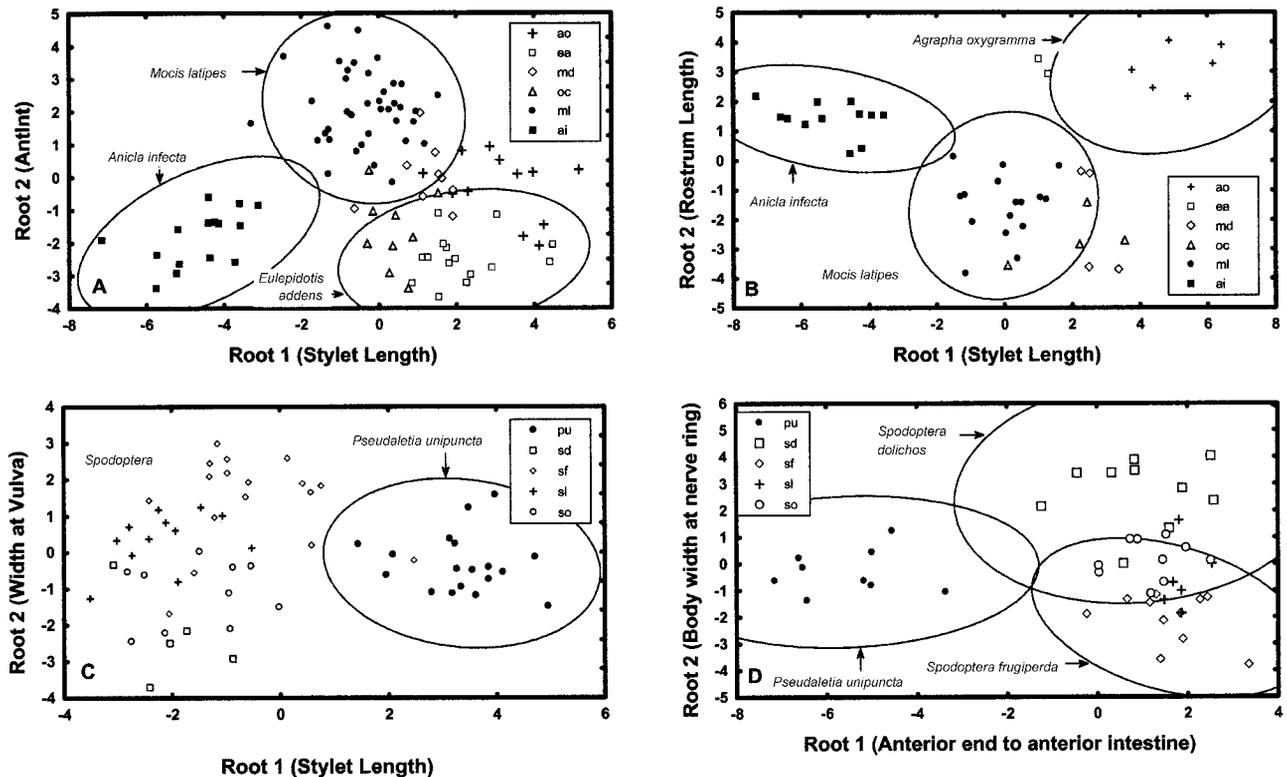


Fig. 2. Plot of discriminant functions for nematode morphometric characters. Ellipses indicate 95% confidence limits for selected host species. A: Female nematodes (Vampyronema) from six host species (Lepidoptera); B: Male nematodes (Vampyronema) from six host species (Lepidoptera). C: Female nematodes (Noctuidonema) from five host species (Lepidoptera). D: Male nematodes (Noctuidonema) from five host species (Lepidoptera). Legend (Host names): ao, *Agrapha oxygramma*; ea, *Anicla infecta*; md, *Mocis disseverans*; ml, *M. latipes*; oc, *Orthodes crenulata*; pu, *Pseudaletia unipuncta*; sd, *Spodoptera dolichos*; sf, *S. frugiperda*; sl, *S. latifascia*; so, *S. ornithogalli*.

Table 4. Summary of forward stepwise discriminant analysis of morphological characters from male and female *Vampyronema* from six species of Noctuidae (Lepidoptera).

Males – <i>Mocis</i> group ¹⁾ (n = 6)				Females – <i>Mocis</i> group ¹⁾ (n = 6)		
Step	Character	F	(df1, df2)	Character	F	(df1, df2)
1	Stylet length	58.07***	(5, 39)	Stylet length	45.73***	(5, 93)
2	Rostrum length	12.19***	(5, 38)	Ant. end to ant. intestine	18.79***	(5, 92)
3	Bulb length	9.29***	(5, 37)	Excretory pore, % of body	18.03***	(5, 91)
4	Ant. end to ant. gonad	8.37***	(5, 36)	G	15.00***	(5, 90)
6	Pharyngeal gland width	5.11**	(5, 34)	Stylet length, % of body	5.73***	(5, 89)
7	Gonad length	4.38**	(5, 33)	V	4.85***	(5, 88)
8	Tail length	4.08**	(5, 32)	Head length	4.76***	(5, 87)
9	Bulb width	2.69*	(5, 31)	Gonad length, % of body	4.45***	(5, 86)
10				Body width at nerve ring	3.55***	(5, 85)

¹⁾ *Mocis* group: *Agrapha oxygramma*, *Anicla infecta*, *Eulepidotis addens*, *Mocis disseverans*, *M. latipes*, *Orthodes crenulata*.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

STEP 3: SIX HOST GROUPS (*VAMPYRONEMA*, NEMATODES WITH SIMPLE SPICULES)

The noctuid moths *Anicla infecta*, *Agrapha oxygramma*, *Eulepidotis addens*, *Mocis disseverans*, *M. latipes* and *Orthodes crenulata* are parasitised by *Vampyronema*, in which the structure of the spicules is simpler and the tail of the females is longer than in *Noctuidonema*. Analysis of morphometric characters of female nematodes from these hosts revealed five significant canonical roots, cumulatively accounting for 100% of the explained variance between groups. The first three roots, representing primarily stylet length, distance from the anterior end to the base of the bulb, and the c ratio, accounted cumulatively for 91.88% of the explained variance between groups. Stylet length was the most important morphological character distinguishing female *Vampyronema* from different hosts (Fig. 2A; Table 4).

Analysis of results of male and female *Vampyronema* on noctuid moths demonstrate the existence of four distinct clades. One of these is *V. dibolia*, the species described from *M. latipes*. The nematodes, particularly the males, on *M. disseverans* are very similar to *V. dibolia* and we consider them to be conspecific. A second clade is formed by nematodes from *A. infecta*, which are well separated from all the other groups (Fig. 2A, B). Stylet length is significantly shorter ($P \leq 0.01$) in nematodes on *A. infecta* than on those from the other hosts. A third *Vampyronema* clade is formed by nematodes on *E. addens*, which differ from *V. dibolia* primarily in the distance from the anterior end to the base of the bulb. The two male nematodes from *E. addens* were also distinct

from other males, an observation in agreement with results from the females. A fourth *Vampyronema* clade is formed by nematodes from *A. oxygramma* (Fig. 2A, B). Although the females appear to be intermediate between those on *M. latipes* and *E. addens*, the males are distinct, based on differences in stylet and rostrum length. Nematodes on *O. crenulata* are also intermediate but males are very similar to *V. dibolia* and we consider them to be conspecific.

In the males, there were five significant canonical roots, represented primarily by stylet length, spicule rostrum length and bulb length, respectively. These accounted cumulatively for 92.9% of the explained variation between males from these six host species. Stylet length was the most important morphological character in defining males (Fig. 2B), although rostrum length was also identified as an important character.

STEP 4: FIVE HOST SPECIES (*NOCTUIDONEMA*, NEMATODES WITH COMPLEX SPICULES)

Discriminant analysis of female nematodes from four *Spodoptera* species and *Pseudaletia* revealed four significant ($P \leq 0.05$) canonical roots. The first two, representing primarily stylet length and body width at the vulva, respectively, accounted for a cumulative proportion of 87.93% of the explained variance between groups. Female nematodes from *Pseudaletia* were well separated from those parasitising *Spodoptera* spp. on the basis of the shorter stylet. Nematodes from the four species of *Spodoptera* were poorly defined and showed a considerable amount of overlap. Even the groups that appeared to

Table 5. Summary of forward stepwise discriminant analysis of morphological characters from male and female *Noctuidonema* from five species of *Spodoptera* and *Pseudaletia*

Step	Males – <i>Spodoptera</i> and <i>Pseudaletia</i> ¹⁾ (n = 5)		Females – <i>Spodoptera</i> and <i>Pseudaletia</i> ¹⁾ (n = 5)	
	Character	F (df1, df2)	Character	F (df1, df2)
1	Ant. end to ant. intestine	25.24*** (4, 41)	Stylet length	39.14*** (4, 57)
2	Body length	14.91*** (4, 40)	Body width at vulva	12.50*** (4, 56)
3	c'	12.23*** (4, 39)	Bulb width	5.91*** (4, 55)
4	Bulb width	7.25*** (4, 38)	a	4.98** (4, 54)
5	Body width at nerve ring	5.00*** (4, 37)	Vulva to anterior end	3.80** (4, 53)
6	Spicule length	4.09*** (4, 36)	Body width at nerve ring	5.79*** (4, 52)
7			Maximum body width	2.65* (4, 51)

¹⁾ *Pseudaletia unipuncta*, *Spodoptera dolichos*, *S. frugiperda*, *S. latifascia*, *S. ornithogalli*

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

be the most different, *S. frugiperda* and *S. dolichos*, were extensively overlapped (Fig. 2D; Table 5).

In the males, there were three significant canonical roots, accounting for 97.64% of the explained variance between groups. The first root represents the distance from the anterior end to the anterior intestine and V about equally, whereas the second root represents primarily the body width at the nerve ring (Fig. 2D; Table 5). Male nematodes from *P. unipuncta* were well separated from nematodes from *Spodoptera*, which agrees with results from the females. Males on *S. dolichos* showed a moderate amount of separation from males on the other three species of *Spodoptera*.

DATA ANALYSIS

Eighteen morphological characters contribute to differentiation among male nematodes on 14 host species included in this study. The most significant and useful of these are the distance of the excretory pore from the anterior end, spicule length and stylet length. These three characters and V are the most useful in differentiating between females (Table 2). Furthermore, ANOVA showed that the stylet length of nematodes on the two species of Sphingidae is significantly greater ($P \leq 0.001$) than the stylet length of nematodes on any of the other host species. The two populations of nematodes from Sphingidae differ significantly in only eight morphological characters, whereas each differs from *V. dibolia* in about 20 characters. Females on *P. lusca* differ from those on *X. chiron* in the longer length of the stylet, greater length and width of the bulb, greater distance of the excretory pore and bulb base from the anterior end, greater body width at the vulva, and greater excretory pore distance as a per-

centage of body length. However, in the absence of males from *X. chiron*, no conclusion can be reached on whether nematodes on Sphingidae are conspecific. The consistent separation of nematodes parasitising Sphingidae from all other Acugutturidae suggests that they probably represent at least one new genus. Particularly remarkable is the long stylet, which represents about 37% of the body length and averages about 190 μm in length (Fig. 3).

Discriminant analysis shows that the five groups of nematodes with complex spicules (*Noctuidonema*) are well separated (Fig. 1C, D) from the six groups with simple spicules (*Vampyronema*). Although the discrete character of spicule structure is sufficient to separate these two genera, particularly good separation can also be made based on important continuously variable characters such as stylet length, distance of the excretory pore to the anterior end, and the V ratio. Furthermore, within each of these two genera, separate groups can be detected, based primarily on differences in stylet length. Thus, the nematodes on *Pseudaletia unipuncta* differ from the nematodes on the four *Spodoptera* spp. (Fig. 1C, D), and those on *Anicla infecta*, *Agrapha oxygramma*, and *Eulepidotis addens* differ from *V. dibolia* and its conspecific on *M. disseverans* (Fig. 2A, B).

Female nematodes on *P. unipuncta* differ ($P \leq 0.05$) from those on *S. dolichos*, *S. frugiperda*, *S. latifascia*, and *S. ornithogalli* in eight, 18, 14, and 14 morphological characters, respectively. These results provide strong support for separate taxonomic status of nematodes on *P. unipuncta* as a new species of *Noctuidonema*. Results from simple scatterplots prepared for each gender using the most important morphological characters identified by discriminant analysis agree with the preceding analysis.

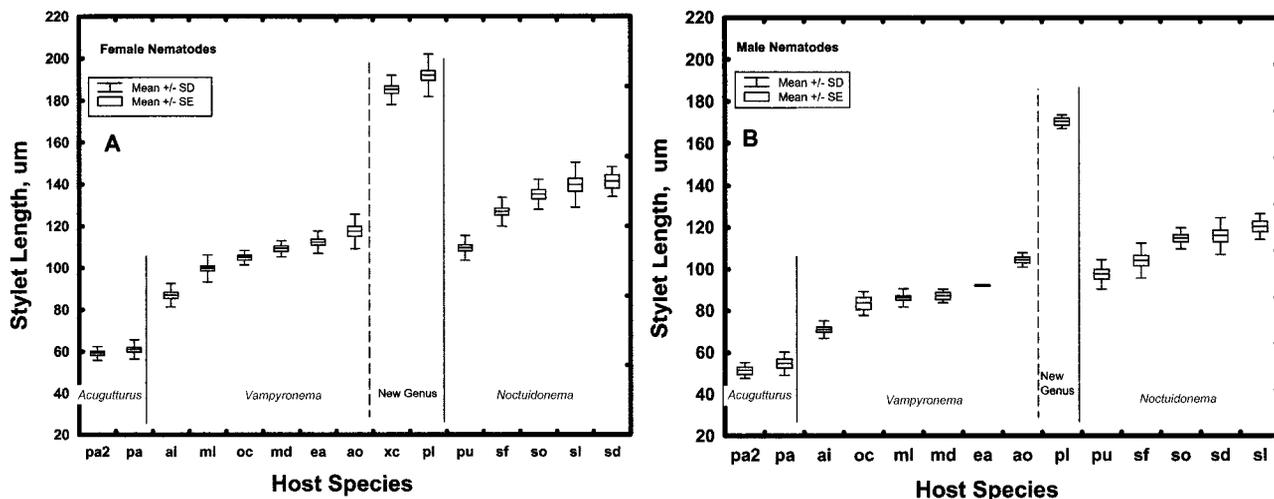


Fig. 3. Plot of stylet length of ectoparasitic nematodes from insect hosts (*Blattodea* and *Lepidoptera*). *A*: Female nematodes from 15 host species. *B*: Male nematodes from 14 host species. Legend (Host names): *ao*, *Agropha oxygramma*; *ai*, *Anicla infecta*; *ea*, *Eulepidotis addens*; *md*, *Mocis disseverans*; *ml*, *M. latipes*; *oc*, *Orthodes crenulata*; *pa*, *Periplaneta americana* (*St Lucia*); *pa2*, *P. americana* (*Guadeloupe*); *pl*, *Perigonia lusca*; *pu*, *Pseudaletia unipuncta*; *sd*, *Spodoptera dolichos*; *sf*, *S. frugiperda*; *sl*, *S. latifascia*; *so*, *S. ornithogalli*; *xc*, *Xylophanes chiron*.

Table 6. Percent correct classification of male nematodes based on posterior probabilities that a case belongs to a particular host species.

Host species	Percent correct classifications – male nematodes				Percent correct classifications – female nematodes					
	n	Hosts 1-15	Hosts 1-11	Hosts 1-6	Hosts 7-11	n	Hosts 1-15	Hosts 1-11	Hosts 1-6	Hosts 7-11
1 <i>Agropha oxygramma</i>	7	100	100	100	–	12	91.7	100	91.7	–
2 <i>Anicla infecta</i>	12	100	100	100	–	15	100	100	100	–
3 <i>Eulepidotis addens</i>	2	100	100	100	–	15	100	100	100	–
4 <i>Mocis disseverans</i>	4	100	75	75	–	9	88.9	88.9	88.9	–
5 <i>Mocis latipes</i>	16	100	100	100	–	39	92.3	97.4	94.8	–
6 <i>Orthodes crenulata</i>	4	100	100	100	–	9	88.9	88.9	88.9	–
7 <i>Spodoptera dolichos</i>	10	100	100	–	90	5	100	100	–	80
8 <i>Spodoptera frugiperda</i>	11	90.9	90.9	–	100	17	76.5	82.4	–	76.5
9 <i>Spodoptera latifascia</i>	6	66.7	83.3	–	50	12	75	83.3	–	66.7
10 <i>Spodoptera ornithogalli</i>	10	100	100	–	100	10	90	90	–	90
11 <i>Pseudaletia unipuncta</i>	9	100	100	–	100	18	100	94.4	–	94.4
12 <i>Perigonia lusca</i>	4	100	–	–	–	19	93.8	–	–	–
13 <i>Periplaneta americana</i> (1)	7	100	–	–	–	18	88.2	–	–	–
14 <i>Periplaneta americana</i> (2)	5	100	–	–	–	13	84.6	–	–	–
15 <i>Xylophanes chiron</i>	0	–	–	–	–	18	100	–	–	–

(1) St Lucia;
 (2) Guadeloupe.

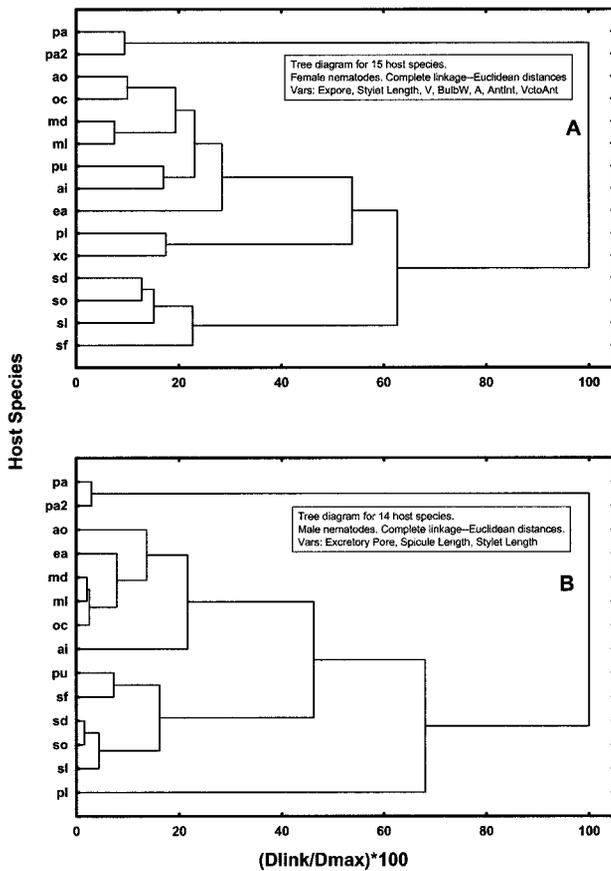


Fig. 4. Tree diagram for ectoparasitic nematodes from insect host species (Blattodea and Lepidoptera). The tree was produced using Euclidean distance as the distance measure and complete linkage as the amalgamation rule. Both graphs are plotted on an X-axis standardised by $(Dlink/Dmax) \cdot 100$, which represents the percentage of the range from the maximum to the minimum distance in the data. A: Female nematodes, data for excretory pore, stylet length, V, bulb width, a, anterior end to anterior intestine, and vulva to anterior end were used; B: Male nematodes, data for excretory pore, spicule length, and stylet length were used. Legend (Host Names): pa, *Periplaneta americana* (St Lucia); pa2, *P. americana* (Guadeloupe); ao, *Agrapha oxygramma*; ai, *Anicla infecta*; ea, *Eulepidotis addens*; md, *Mocis disseverans*; ml, *M. latipes*; oc, *Orthodes crenulata*; pl, *Perigonia lusca*; pu, *Pseudaletia unipuncta*; sd, *Spodoptera dolichos*; sf, *S. frugiperda*; sl, *S. latifascia*; so, *S. ornithogalli*; xc, *Xylophanes chiron*.

Spicule length was not an important character in distinguishing among male *Noctuidonema*. Male nematodes on *S. dolichos* and *S. frugiperda* showed the most separation, with moderate overlap, whereas males on *S. ornithogalli* appeared to be most similar to those on *S.*

dolichos and males on *S. latifascia* appeared most similar to those on *S. frugiperda*. Results from both genders agree that nematodes on *Pseudaletia* are different from those on *Spodoptera* and that the nematodes on *S. dolichos* and *S. frugiperda* are the most different, with the nematodes on *S. latifascia* and *S. ornithogalli* being intermediate between these two. Overall, the results do not support the existence of separate species of *Noctuidonema* on hosts of the genus *Spodoptera* (Fig. 4; Table 6).

These results provide evidence that nematodes on *A. infecta*, *A. oxygramma* and *E. addens* are different from each other and from the remaining groups having simple spicules. Nematodes on *M. disseverans* and *O. crenulata* were not distinct from *V. dibolia*, the species parasitising *M. latipes*. Nematodes on *M. disseverans* differed significantly from *V. dibolia* in only six morphological characters in the females and ten in the males. The differences were small and can be attributed to adaptation of conspecific nematodes to parasitism of closely related sympatric host species.

Comparison of the description of *V. daptria* to measurements of other *Vampyronema* in this study indicates that it differs in stylet length and spicule shape. *V. daptria* has the shortest stylet length observed in the genus, ranging from 53–60 μm in males and 59–69 μm in females, which is shorter than the stylet length of nematodes on *A. infecta* (Fig. 3). In addition, spicules of *V. daptria* taper to fine points, whereas in *Vampyronema* from other hosts the spicule tips are blunt. *V. daptria* on *L. porcia* appears to be a distinct species and we therefore do not assign any of the *Vampyronema* from this study to it.

Nematodes on the St Lucia and Guadeloupe populations of *Periplaneta americana* are not well differentiated from each other based on the suite of characters that were examined (Fig. 1A, B). Although there are a few significantly different characters between the two populations, the differences probably do not represent interspecific variation. In addition, the characters of both populations are in agreement with the published description of *Acugutturus parasiticus* (Hunt, 1980).

Examination of additional specimens of ectoparasitic nematodes from other species of Lepidoptera showed that most were *Vampyronema*, whereas *Noctuidonema* was found only on moths from two genera, *Pseudaletia* and *Spodoptera* (Table 7).

Table 7. Association of quadrifine and trifine Noctuidae with *Vampyronema* and *Noctuidonema*

Host Taxon	Hind Wing Venation ¹⁾	Locality ²⁾	Nematodes	Genus
Noctuidae				
Catocalinae				
<i>Anomis oedema</i> Guenée	quadrifine	FG	4♀, 1♂	<i>Vampyronema</i>
<i>Baniana</i> sp.	quadrifine	FG	1♀, 0♂	<i>Vampyronema</i>
<i>Baniana ostia</i> Druce	quadrifine	FG	7♀, 0♂	<i>Vampyronema</i>
<i>Euglyphis</i> sp.	quadrifine	FG	1♀, 0♂	<i>Vampyronema</i>
<i>Eulepidotis addens</i> (Walker)	quadrifine	GU	5♀, 2♂	<i>Vampyronema</i>
<i>Itomia opisthographa</i> (Guenée)	quadrifine	FG	5♀, 1♂	<i>Vampyronema</i>
<i>Lesmone formularis</i> (Geyer)	quadrifine	FG	1♀, 0♂	<i>Vampyronema</i>
<i>Lesmone porcia</i> (Stoll)	quadrifine	GU	– ³⁾	<i>Vampyronema</i> ³⁾
<i>Melipotis fasciolaris</i> (Hübner)	quadrifine	FG	0♀, 1♂	<i>Vampyronema</i>
<i>Metalectra</i> sp.	quadrifine	FG	4♀, 10♂	<i>Vampyronema</i>
<i>Metria</i> sp.	quadrifine	FG	0♀, 1♂	<i>Vampyronema</i>
<i>Mocis diffluens</i> (Guenée)	quadrifine	FG	1♀, 1♂	<i>Vampyronema</i> ⁴⁾
<i>Mocis disseverans</i> (Walker)	quadrifine	GA	9♀, 4♂	<i>Vampyronema</i> ⁴⁾
<i>Mocis latipes</i> Guenée	quadrifine	GA	9♀, 16♂	<i>Vampyronema</i> ⁴⁾
<i>Perasia ora</i> (Cramer) ⁵⁾	quadrifine	FG	10♀, 0♂	<i>Vampyronema</i>
<i>Selenisa</i> sp.	quadrifine	FG	4♀, 0♂	<i>Vampyronema</i>
<i>Zale fictilis</i> (Guenée)	quadrifine	FG	4♀, 1♂	<i>Vampyronema</i>
Plusiinae				
<i>Agrapha oxygramma</i> (Geyer)	quadrifine	GA	13♀, 7♂	<i>Vampyronema</i>
<i>Argyrogramma verruca</i> (Fabricius)	quadrifine	FG	0♀, 2♂	<i>Vampyronema</i>
<i>Chrysoideixis chalcites</i> (Esper)	quadrifine	Fiji	8♀, 7♂	<i>Vampyronema</i>
Amphipyridae				
<i>Spodoptera androgea</i> (Stoll)	trifine	FG	4♀, 0♂	<i>Noctuidonema</i> ⁶⁾
<i>Spodoptera dolichos</i> (Fabricius)	trifine	GA	5♀, 10♂	<i>Noctuidonema</i> ⁶⁾
<i>Spodoptera frugiperda</i> (J.E. Smith)	trifine	GA	17♀, 11♂	<i>Noctuidonema</i> ⁶⁾
<i>Spodoptera latifascia</i> (Walker)	trifine	GA	12♀, 6♂	<i>Noctuidonema</i> ⁶⁾
<i>Spodoptera litura</i> (Fabricius)	trifine	Fiji	15♀, 7♂	<i>Noctuidonema</i> ⁶⁾
<i>Spodoptera ornithogalli</i> (Guenée)	trifine	GA	10♀, 10♂	<i>Noctuidonema</i> ⁶⁾
Hadeninae				
<i>Orthodes crenulata</i> (Butler)	trifine	GA	9♀, 4♂	<i>Vampyronema</i>
<i>Pseudaletia unipuncta</i> (Haworth)	trifine	GA	18♀, 9♂	<i>Noctuidonema</i>
Noctuinae				
<i>Anicla infecta</i> (Ochsenheimer)	trifine	GA	15♀, 12♂	<i>Vampyronema</i>
<i>Tandilia rodea</i> (Schaus)	trifine	FG	2♀, 3♂	<i>Vampyronema</i>
Pyralidae				
Pyraustinae				
<i>Lamprosema mocalis</i> Schaus	–	FG	4♀, 0♂	<i>Vampyronema</i>
Sphingidae				
Macroglossinae				
<i>Erinnys obscura</i> (Fabricius)	–	FG	22♀, 2♂	<i>Vampyronema</i> ⁷⁾
<i>Perigonia lusca</i> Fabricius	–	GU	19♀, 4♂	<i>Vampyronema</i> ⁷⁾
<i>Xylophanes chiron</i> Drury	–	GU	8♀, 0♂	<i>Vampyronema</i> ⁷⁾

¹⁾ Quadrifine and trifine hindwing venation applies to Noctuidae only; Pyralidae and Sphingidae are included for completeness; the status of Plusiinae as quadrifines is ambiguous because it groups together mostly species having quadrifine wing venation yet is often included with chiefly trifine subfamilies (Kitching & Rawlins, 1998). ²⁾ Fiji: Fiji Islands; FG: French Guiana; GU: Guadeloupe; GA: Georgia, USA. ³⁾ *V. daptria* Anderson & Laumond, 1992; designation based on Hunt (1993). ⁴⁾ *V. dibolia*. ⁵⁾ Synonymous with *Nymbis arcuata* Walker (in Rogers *et al.*, 1990a and Simmons & Rogers, 1996). ⁶⁾ *N. guyanense*. ⁷⁾ Probably represents an undescribed genus.

Discussion

The position of the excretory pore posterior to the bulb, the comparatively short length of the stylet, the comparatively long tail, and the simple rose-thorn-shaped spicules are sufficient to clearly separate *Acugutturus*, a parasite of Blattodea, from *Vampyronema* and *Noctuidonema*, parasites of Lepidoptera. Sampled populations reveal four inferred monophyletic groups: *Acugutturus parasiticus* from two West Indian populations of *Periplaneta americana*; a new genus on Sphingidae; *Noctuidonema guyanense* from *Spodoptera* spp., with nematodes on *P. unipuncta* as a probable new species of *Noctuidonema*; and *Vampyronema* spp. on the remaining hosts in this study, with the nematodes on *A. infecta*, representing a new species of *Vampyronema*.

Each inferred monophyletic group can be defined by several unique combinations of characters, quantitative and qualitative, that satisfy the minimal requirements for species diagnosis (Wheeler, 1999; Wheeler & Platnick, 2000). Such characters appear to represent lineage independence *via* autapomorphies, hypotheses ideally tested *via* outgroup comparison. Such tests, while more philosophically satisfying (Adams, 1998; Wiley & Mayden, 2000), are beyond the scope of the present paper.

According to Kitching and Rawlins (1998), differences in hindwing venation broadly divide the Noctuidae into two phenetic groups, trifines and quadrifines. Catocalinae and Plusiinae are quadrifines (but see comment 1 in Table 7), whereas the other noctuid subfamilies mentioned in this study are trifines. Complex spicules probably represent a derived condition found thus far only in nematodes on two genera of trifine noctuids, *Pseudaletia* and *Spodoptera*, while the simple spicules of *Vampyronema* probably represent a plesiomorphic state found in both trifine and quadrifine noctuids. The extremely long stylets of nematodes from Sphingidae may have arisen in response to parasitisation of large moths.

The Acugutturidae are associated with two very distantly related insect orders, Blattodea and Lepidoptera (Endopterygota). There is no record of occurrence of the Acugutturidae on any other insect order. Among the Lepidoptera, the Acugutturidae, and more precisely the Noctuidonematinae, are known from only six families: Geometridae, Lasiocampidae, Noctuidae, Notodontidae, Pyralidae, and Sphingidae (Marti *et al.*, 2000), most of which are not closely related, with the exception of Lasiocampidae and Sphingidae, members of the Bombycoidea (and their relatives) clade (Lemaire & Minet, 1998), and

Notodontidae and Noctuidae, members of the Noctuoidea superfamily (Kitching & Rawlins, 1998).

One explanation for such a surprising distribution of the Acugutturidae on their hosts may be that available data are too sparse, due to insufficient sampling of other lepidopteran families. While this may be true, the study conducted by Marti *et al.* (2000) shows that there is a clear specificity in nematode host preference. Marti *et al.* (2000) updated the list of lepidopteran hosts of Noctuidonematinae. It seems clear that although many Lepidoptera species belonging to 11 families and 115 genera have been examined for nematodes, only members of the six aforementioned obtectomeran (Kristensen & Skalski, 1998) families have been found to be positive for Noctuidonematinae. With the exception of Pyralidae, all these families are members of the Macrolepidoptera clade. Moreover, the positive Noctuidae species represent nearly 90% of the total positive species found. Therefore, we cannot state that the Noctuidonematinae are not potentially parasites of most of the lepidopteran families, but we can state that, at least in the Neotropical Region, they parasitise the Macrolepidoptera preferentially, and among that clade, mostly a single family, the Noctuidae (Marti *et al.*, 2000).

The Aphelenchida include several different trophic types, with many species being mycetophagous or phytoparasitic, with about one third of known species being entomoparasitic (Hunt, 1993). Considering the list of insect hosts, we cannot hypothesise that the Acugutturidae are derived from a single ancestor transferring from a plant to an insect host. It seems more realistic to propose several transfers from plant to insect hosts during the history of the Acugutturidae. A transfer (Acugutturinae) occurred on the Blattodea and another one (Noctuidonematinae) on the Lepidoptera. Another transfer may have occurred on the Bombycoidea *sensu lato*. If at least two such principal transfers occurred, then it is possible that the Acugutturinae and Noctuidonematinae would not be confamilial and that these two taxonomic units deserve familial rank. Cladistic and molecular phylogenetic analysis will help to solve that problem. The existence of symbiotic bacteria in these nematodes (Marti *et al.*, 1995) should provide an independent means of analysing these relationships.

Concerning the relationship between Lepidoptera and its ectoparasitic nematodes, the present work highlights the following situation: a new genus of Noctuidonematinae parasitises Sphingidae, a genuine Bombycoidea (Lemaire & Minet, 1998); *Vampyronema* parasitises the

quadrifine noctuid subfamily Catocalinae (*sensu lato*, including Ophiderinae), Plusiinae, an Hadeninae, and a Noctuidae (both typical trifine noctuids); and *Noctuidonema* spp. parasitises another Hadeninae and several *Spodoptera*, a trifine noctuid genus previously included in the Amphipyridae, but now of uncertain subfamilial affiliation (Poole, 1995; Kitching & Rawlins, 1998). Except for nematodes on Sphingidae, which likely correspond to a well-separated clade whose phylogenetic relationship to other Noctuidonematinae needs to be specified, it appears that *Vampyronema* and *Noctuidonema* both parasitise Noctuidae but that *Vampyronema* parasitises both quadrifine and trifine noctuids. *Noctuidonema*, possibly a derived form of Noctuidonematinae, is restricted to a few genera (*Spodoptera* and *Pseudaletia*) of trifine noctuids that are not closely related (Mitchell *et al.*, 2000).

Recent work has questioned the monophyly of the Noctuidae and there is a growing feeling among molecular taxonomists that the Noctuidae may be paraphyletic, several quadrifine subfamilies, including Catocalinae, being more closely related to Arctiidae and Lymantriidae than to trifine noctuids (Weller *et al.*, 1994; Mitchell *et al.*, 1997; Fang *et al.*, 2000; Mitchell *et al.*, 2000). The latter group is considered as monophyletic. Clearly, systematics of the Noctuidonematinae do not match the tentative new classification of the Noctuidae.

This lack of congruence between parasite and host systematics leads us to consider that the relationships between Acugutturidae and their hosts have not been guided by coevolutionary forces in the sense of Ehrlich and Raven (1964), but by horizontal transfers (Rogers & Marti, 1996) and sequential evolution (Jermy, 1984; Futuyma & McCafferty, 1990). The Acugutturidae, and especially the Noctuidonematinae, have likely experienced an adaptive radiation on higher Lepidoptera long after their host diversification. History of the Noctuidonematinae has been characterised by host transfer events onto fairly distantly related hosts, followed by adaptation to new hosts leading to speciation processes that now permit us to distinguish easily fairly species-specific populations or strains of *Vampyronema* or *Noctuidonema*. In moths, these host transfers may have occurred through contamination of nematode-free species by nematode eggs, juveniles, or adults left on resting or feeding surfaces (leaves or flowers) by individuals of infested species. The success of such contamination may be linked to the availability and accessibility of host intersegmental membranes thin enough to be pierced by nematode stylets, a factor that

may have physically and dramatically restricted the host range of all Acugutturidae.

The present morphological analysis of acugutturid nematodes parasitising 13 species of Lepidoptera and one species of cockroach revealed a greater degree of diversity than previously known in this group. The genus *Vampyronema*, grouping nematodes with simple spicules, includes two inferred new species in addition to *V. dibolia* and *V. daptria* already known from the Lepidoptera. The genus *Noctuidonema*, grouping nematodes of Lepidoptera with complex spicules, includes a new species, and the nematodes found on Sphingidae probably belong to a new genus. The relationship between these nematodes and their hosts appears to be much more complex than previously thought. In addition to their benefits as parasites of pest Lepidoptera, acugutturid nematodes are an interesting model for parasite-host coevolutionary studies.

Acknowledgements

We thank David Hunt (CABI Bioscience, UK), Daniel Lachaise (CNRS, France), Alvin Simmons (USDA, Charleston, SC, USA), and S. Patricia Stock (University of California, Davis, CA, USA) for reviewing earlier versions of this paper. We thank Jeanette Williams and John Clayton for collecting cockroaches in St Lucia and moths in Fiji, respectively. We thank the following individuals for identifying or confirming our identifications of insects: David Nickle (Smithsonian, Washington, DC, USA) for *Periplaneta americana*, Pierre Zagatti (INRA, France) for Pyralidae, and Hugo Kons (University of Florida, Gainesville, FL, USA) for *Orthodes crenulata*.

References

- ADAMS, B.J. (1998). Species concepts and evolutionary paradigm in modern nematology. *Journal of Nematology* 30, 1-21.
- ANDERSON, R.V. & LAUMOND, C. (1990). *Noctuidonema guyanense* Remillet & Silvain, 1988: morphologie du spicule, redescription du mâle et diagnose amendée du genre *Noctuidonema*. *Revue de Nématologie* 13, 433-436.
- ANDERSON, R.V. & LAUMOND, C. (1992). *Noctuidonema daptria* n. sp. (Nematoda: Aphelenchoididae), an ectoparasite of the moth *Lesmone porcia* (Stoll). *Journal of Nematology* 24, 16-22.
- EHRlich, P. & RAVEN, P. (1964). Butterflies and plants: a study in evolution. *Evolution* 18, 586-608.
- FANG, Q.Q., MITCHELL, A., REGIER, J.C., MITTER, C., FRIEDLANDER, T.P. & POOLE, R.W. (2000). Phylogenetic

- utility of the nuclear gene dopa decarboxylase in noctuid moths (Insecta: Lepidoptera: Noctuoidea). *Molecular Phylogenetics and Evolution* 15, 473-486.
- FOOTIT, R.G. & SORENSEN, J.T. (1992). Ordination methods: their contrast to clustering and cladistic techniques. In: Sorensen, J.T. & Footit, R.G. (Eds). *Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationals*. Amsterdam, The Netherlands, Elsevier Science Publishers, pp. 1-10.
- FUTUYMA, D. & MCCAFFERTY, S. (1990). Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera, Chrysomelidae). *Evolution* 44, 1885-1913.
- HUNT, D. (1980). *Acugutturus parasiticus*, n. g., n. sp., a remarkable ectoparasitic aphelenchoid nematode from *Periplaneta americana* (L.), with proposal of Acugutturinae n. subf. *Systematic Parasitology* 1, 167-170.
- HUNT, D. (1993). *Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics*. Wallingford, UK, CAB International, 352 pp.
- JERMY, T. (1984). Evolution of insect/host plant relationships. *The American Naturalist* 124, 609-630.
- KITCHING, I.J. & RAWLINS, J.E. (1998). The Noctuoidea. In: Kristensen, N.P. (Ed.). *Vol. 1: Evolution, systematics and biogeography, Part 35, Lepidoptera: moths and butterflies. Handbuch der Zoologie*. Berlin, Germany, Walter de Gruyter, pp. 355-401.
- KRISTENSEN, N.P. & SKALSKI, A.W. (1998). Phylogeny and paleontology. In: Kristensen, N.P. (Ed.). *Vol. 1: Evolution, systematics and biogeography, Part 35, Lepidoptera: moths and butterflies. Handbuch der Zoologie*. Berlin, Germany, Walter de Gruyter, pp. 7-25.
- LEMAIRE, C. & MINET, J. (1998). The Bombycoidea and their relatives. In: Kristensen, N.P. (Ed.). *Vol. 1: Evolution, systematics and biogeography, Part 35, Lepidoptera: moths and butterflies. Handbuch der Zoologie*. Berlin, Germany, Walter de Gruyter, pp. 321-353.
- MARTI, O.G., JR & ROGERS, C.E. (1995). *Noctuidonema dibolia*, n. sp. (Aphelenchida: Acugutturidae), an ectoparasite of the moth *Mocis latipes* (Lepidoptera: Noctuidae). *Journal of Nematology* 27, 387-394.
- MARTI, O.G., JR & ROGERS, C.E. (2000). Effect of *Noctuidonema guyanense* (Nematoda: Acugutturidae) infection on the longevity of feral male *Spodoptera frugiperda* (Lepidoptera: Noctuidae) moths. *Journal of Entomological Science* 35, 259-266.
- MARTI, O.G., JR, ROGERS, C.E., SILVAIN, J.-F. & SIMMONS, A.M. (1990). Pathological effects of an ectoparasitic nematode *Noctuidonema guyanense* (Nematoda: Aphelenchoididae) on adults of the fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 83, 956-960.
- MARTI, O.G., JR, ROGERS, C.E. & STYER, E.L. (1995). Report of an intracellular bacterial symbiont in *Noctuidonema guyanense*, an ectoparasitic nematode of *Spodoptera frugiperda*. *Journal of Invertebrate Pathology* 66, 94-96.
- MARTI, O.G., JR, LALANNE-CASSOU, B., SILVAIN, J.-F., KERMARREC, A. & SIMMONS, A.M. (2000). Ectoparasitic nematodes (Aphelenchoidoidea: Acugutturidae) of Lepidoptera and Blattodea in Guadeloupe. *Nematology* 2, 669-684.
- MITCHELL, A., CHO, S., REGIER, J.C., MITTER, C., POOLE, R.W. & MATTHEWS, M. (1997). Phylogenetic utility of elongation factor-1 in Noctuoidea (Insecta: Lepidoptera): the limits of synonymous substitution. *Molecular Biology and Evolution* 14, 381-390.
- MITCHELL, A., MITTER, C. & REGIER, J.C. (2000). More taxa or more characters revisited: combining data from nuclear protein-encoding genes for phylogenetic analyses of Noctuoidea (Insecta: Lepidoptera). *Systematic Biology* 49, 202-224.
- POOLE, R.W. (1995). Noctuoidea. Noctuidae (part). Cucullinae, Stiriinae, Pasaphidinae (part). In: *The moths of America north of Mexico including Greenland*. Washington, DC, USA, The Wedge Entomological Research Foundation, Fascicle 26, 1246 pp.
- REMILLET, M. & SILVAIN, J.-F. (1988). *Noctuidonema guyanense*, n. g., n. sp., (Nematoda: Aphelenchoididae) ectoparasite de noctuelles du genre *Spodoptera* (Lepidoptera: Noctuidae). *Revue de Nématologie* 11, 21-24.
- ROGERS, C.E. & MARTI, O.G., JR (1992). *Noctuidonema guyanense* (Nematoda: Aphelenchoididae): Population profiles on male and female fall armyworm moths. *Journal of Entomological Science* 27, 354-360.
- ROGERS, C.E. & MARTI, O.G., JR (1993). Infestation dynamics and distribution of *Noctuidonema guyanense* (Nematoda: Aphelenchoididae) on adults of *Spodoptera frugiperda* and *Mocis latipes* (Lepidoptera: Noctuidae). *Florida Entomologist* 76, 326-333.
- ROGERS, C.E. & MARTI, O.G., JR (1994). Population structure and transfer success of *Noctuidonema guyanense* (Nematoda: Aphelenchoididae) on moths of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of the Entomological Society of America* 87, 327-330.
- ROGERS, C.E. & MARTI, O.G., JR (1996). Beet armyworm (*Spodoptera exigua*) as a host for the ectoparasitic nematode, *Noctuidonema guyanense*. *Journal of Agricultural Entomology* 13, 81-86.
- ROGERS, C.E., MARTI, O.G., JR, SIMMONS, A.M. & SILVAIN, J.-F. (1990a). Host range of *Noctuidonema guyanense* (Nematoda: Aphelenchoididae): an ectoparasite of moths in French Guiana. *Environmental Entomology* 19, 795-798.
- ROGERS, C.E., SIMMONS, A.M. & MARTI, O.G., JR (1990b). Parasitism of Lepidoptera adults by *Noctuidonema guyanense* Remillet and Silvain (Nematoda: Aphelenchoididae) in southeastern United States. *Journal of Agricultural Entomology* 7, 242-245.

- ROGERS, C.E., MARTI, O.G., JR & CLAYTON, J.A. (1997). Report of the ectoparasitic nematode *Noctuidonema guyanense* (Acugutturidae) infesting Lepidoptera in the Fiji Islands. *Nematologica* 43, 505-506.
- SANMARTÍN, I. & MARTÍN-PIERA, F. (1999). A morphometric approach to the taxonomy of the genus *Ceramida* (Coleoptera: Scarabaeoidea: Melolonthidae). *The Canadian Entomologist* 131, 573-592.
- SIMMONS, A.M. & ROGERS, C.E. (1994). Effect of an ectoparasitic nematode, *Noctuidonema guyanense*, on adult longevity and egg fertility in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biological Control* 4, 285-289.
- SIMMONS, A.M. & ROGERS, C.E. (1996). Ectoparasitic acugutturid nematodes of adult Lepidoptera. *Journal of Nematology* 28, 1-7.
- WELLER, S.J., PASHLEY, D.P., MARTIN, J.A. & CONSTABLE, J.L. (1994). Phylogeny of noctuid moths and the utility of combining independent nuclear and mitochondrial genes. *Systematic Biology* 43, 194-211.
- WHEELER, Q.D. (1999). Why the phylogenetic species concept? — Elementary. *Journal of Nematology* 31, 134-141.
- WHEELER, Q.D. & PLATNICK, N.I. (2000). The phylogenetic species concept (*sensu* Wheeler and Platnick). In: Wheeler, Q.D. & Meier, R. (Eds). *Species concepts and phylogenetic theory: a debate*. New York, NY, USA, Columbia University Press, pp. 55-69.
- WILEY, E.O. & MAYDEN, R.L. (2000). The evolutionary species concept. In: Wheeler, Q.D. & Meier, R. (Eds). *Species concepts and phylogenetic theory: a debate*. New York, NY, USA, Columbia University Press, pp. 70-89.