Community structure and ecological niches of free-living terrestrial nematodes in north-eastern Vietnam

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Zusammenfassung

Nematoden sind hochabundant und global die diverseste Gruppe der Bodenfauna. Es gibt nur wenige Taxonomen für freilebende Nematoden und daher existieren weltweit nur eine Handvoll Studien zu freilebenden Nematoden aus tropischen Gebieten. Der Artenreichtum und die funktionelle Zusammensetzung von Nematodengemeinschaften In Vietnam sind noch vollständig unbekannt. Veränderungen von Bodenfunktionen beeinflussen Nematodengemeinschaften lange bevor chemische und physikalische Bodenparameter messbar sind. Daher sind Nematoden exzellente Indikatoren von Bodenfunktionen und Ökosystemdienstleistungen.

Wir untersuchten die Nematodenfauna in Böden dreier Naturreservate in den Provinzen Cao Bang, Lang Son und Cat Ba Provinces im Nordosten Vietnams im May 2013. Nematoden wurden (wenn möglich) auf auf Artniveau bestimmt um die Zahl und Diversität der bekannten und unbekannten Taxa zu bestimmen. Basierend auf den trophischen Gruppen wurden die Nematoden anschließend funktionelle Gilden zugeordnet. Mit diesen Daten wurden unterschiedliche ökologische Indices errechnet, um zu beschreiben wie Änderungen in der Landnutzung die Bodenfunktionen in Primärwäldern, Sekundärwäldern, Brandrodung und intensiv bewirtschafteten Akerflächen beeinflussten.

Insgesamt wurden mehr als 14 000 Individuen identifiziert. Diese Nematoden gehörten zu 105 Genera, 42 Familien, und 9 Ordnungen. Davon gehörten 48 Genera und 13 Familien allein zu der Gruppe der Dorylaimida. Die taxonomische Bearbeitung der Ordnung Dorylaimda ist besonders anspruchsvoll da sie hochdivers ist, aber sich die einzelnen Arten morphologisch wenig unterscheiden. Mehr als 1/3 der identifizierten Arten der Dorylaimiden waren noch nicht beschrieben. Insgesamt wurden 12 neue Arten beschrieben, davon 3 Arten im Genus *Aporcelaimoides*: *A. brevistylum, A. minor, A. silvaticum;* 3 Arten im Genus *Aporcelinus*: *A. falcicaudatus, A. paramamillatus, A. paraseychellensis,* und 6 Arten im Genus *Sectonema*: *S. birrucephalum, S. buccociliatum, S. caobangense, S. ciliatum*, *S. tropicum* und *S. vietnamense.* Drei Arten konnten zum ersten Mal in Vietnam nachgewiesen werden: *Axonchium thoubalicum, Belondira murtazai and Oxybelondira paraperplexa.* Eine Art in der *Cephalobus topali* Gruppe wurde neubeschrieben und zu *Acrobeloides* transferiert. Molekulare Daten bestätigten die geringe Kenntnis der Dorylaimiden; nur 5 Sequenzen aus der Genbank Datei wiesen mehr als 95% Ähnlichkeit zu den sequenzierten Nematoden auf.

Die Nematodengemeinschaften unterschieden sich sigfnifikant zwischen den Regionen und den Landnutzungsintensitäten. Nematodengemeinschaften von Primärwäldern beinhalteten vor allem große, langlebige Prädatoren und Omnivore (46-74%), im Vergleich zu einer kontinuierlichen Zunahme von kurzlebigen Bacterivoren (5-15%), Pflanzenparasiten (11-24%)

und Pilzfressern (3-15%) mit Zunahme der Landnutzung. Die Analyse der Nematodengemeinschaften erwies sich damit als nützlich um funktionelle Veränderungen im Bodenökosystem vorherzusagen. Unterschiedliche ökologische Indices (der Maturity Index (MI), der Plant-Parasite Index (PPI), der Basal Index, Enrichment index, Structure index und Channel Index) wurden in temperierten Ökosystemen entwickelt um anhand der Veränderungen in der trophischen Zusammensetzung von Nematodengemeinschaften Änderungen in Bodenfunktionen und Ökosystemdienstleistungen zu ermitteln. Wir testeten ob dieselben Rahmenbedingungen auf tropische Böden angewandt werden können. Insbesondere die Maturity Indices ließen sich auf tropische Böden kaum anwenden. Darüber hinaus zeigte sich, dass vergleichbare Veränderungen in der Landnutzung zu ganz unterschiedlichen Veränderungen der Böden führten. Zum Beispiel veränderten sich die C/N Verhältnisse nicht wie erwartet mit steigender Landnutzung und auch Veränderungen der Bodenmikroflora waren nicht mit der Landnutzung korreliert.

Insgesamt haben unsere Untersuchungen sehr zum Verständnis der Funktion und den Ursachen der Degeneration tropischer Böden, als auch zur Kenntnis der Biodiversität der freilebenden Nematoden beigetragen. Diese Studie fand klare Belege für eine höheren Diversität und einen höheren Artenreichtums der Nematoden in tropischen Regionen im Vergleich zu temperierten Regionen. Zusammenfassend haben meine Arbeiten über freilebende Nematodengemeinschaften in Vietnam gezeigt, dass 1) die Diversität der Nematodengemeinschaften in tropischen Böden stark unterschätzt wurde, 2) Veränderungen der Landnutzung sich stark auf die Nematodengemeinschaften auswirkten, und 3) Veränderungen der Nematodengemeinschaften entlang von Landnutzungsgradienten nicht einfach durch abiotische Bodenfaktoren erklärt werden konnten.

Summary

Nematodes are highly abundant and globally the most diverse group of soil fauna. Due to the lack of taxonomists, only a handful of studies exist on communities of free-living nematodes in tropical areas worldwide. The true species richness and functional composition of nematode communities in Vietnam are virtually unknown. Changes in soil functioning affect nematode community composition long before adverse effects on chemical and physical soil parameters are measurable. Consequently, nematodes are excellent indicators of soil function and ecosystem services.

We investigated the nematode fauna in soils of three Nature Reserves in Cao Bang, Lang Son and Cat Ba provinces in north-eastern Vietnam in May, 2013. First, we identified the nematodes (if possible) until species level to estimate the numbers and diversity of known and undescribed taxa. Then, based on the different trophic groups, nematode functional guilds were determined. These data were used to calculate different ecological indices in order to describe how changes in land use affected the functioning of soils in a gradient from primary forest, secondary forests, slash and burn agriculture and intensive agricultural management.

In total, more than **14,000** specimens were identified. These nematodes belonged to **105 genera**, **42 families** and **9 orders,** of which **48 genera** and **13 families** belonged solely to the order Dorylaimida. The Order Dorylaimida is taxonomically particularly challenging because it is highly diverse, but species show only little differences in morphological characters. More than 1/3 of the identified species of Dorylaims were new to science. Twelve new species were described comprising three species of the genus *Aporcelaimoides*: *A. brevistylum, A. minor, A. silvaticum;* three species of the genus *Aporcelinus*: *A. falcicaudatus, A. paramamillatus, A. paraseychellensis,* and six species of the genus *Sectonema*: *S. birrucephalum, S. buccociliatum, S. caobangense, S. ciliatum*, *S. tropicum* and *S. vietnamense.* Three species were recorded for the first time from Vietnam: *Axonchium thoubalicum, Belondira murtazai and Oxybelondira paraperplexa.* One species *Cephalobus topali* was re-described and transferred to the genus *Acrobeloides*. Also molecular data confirmed that the diversity of dorylaims is little known, only 5 sequences obtained from Genbank could be assigned to the sequenced nematodes with a similarity higher than 95%.

The nematode assemblages and diversity were significantly different between different regions and among four land use intensities. Nematode assemblages in primary forests were composed mainly of large and long-lived predators and omnivores (46-74%), compared to a successive increase of short-lived bacterivores (5-14%), plant parasites (11-24%) and fungivores (3-15%) with land-use intensification. Our data indicated that nematode

community analysis was a useful tool to predict functional changes in soil ecosystems. Different ecological indices (i.e. the Maturity Index (MI), the Plant-Parasite Index (PPI), the Basal Index, Enrichment index, Structure index and Channel Index) have been developed in temperate ecosystems to describe shifts in soil functioning and ecosystem services based on changes in the composition of nematode trophic groups. We tested whether this framework could be applied to tropical soils. In particular the Maturity indices were hardly applicable in tropical soils. Further it turned out that comparable change in land use led to very different transformations of soil conditions. For example the soil C/N ratios did not change as expected with increasing land-use and were not correlated with the composition of the soil microflora.

Overall, our studies substantially contribute to our understanding of the functioning and the causes of degradation of tropical soils and to the knowledge of the biodiversity of free-living nematodes. This study found clear evidence corroborating the idea that the diversity and species richness of nematodes from tropical regions is higher than temperate regions. In conclusions, my studies of free-living nematodes communities of Vietnam showed: 1) The diversity of nematodes in tropical soils has been vastly underestimated; 2) Land-use intensity had strong impacts on nematode communities; 3) Changes in nematode communities along gradients of within increasing land use intensity could not be simply explained by abiotic soil conditions.

1. Introduction

1.1 Nematodes and their role in the environment

1.1.1 Nematodes as a zoological group

Nematodes (phylum Nematoda), are rounded, non-segmented worms, but despite their comparatively simple body plan they evolved a very high taxonomic and functional diversity. Free-living nematodes are found in the soil, freshwater or in the sea. They occur in virtually all environments and are able to survive under extreme conditions. They are often highly abundant and in grassland one square metre may contain twenty million nematodes (Overgaard Nielsen 1949). In a square metre of littoral sand, Wierser (1956) found ten million specimens. In a hectare of midland forest in Hungary, Andrássy (1955) estimated around seventy billion nematodes. In addition to free-living nematodes, there are many parasitic nematodes. They parasite nearly all groups of animals and plants, and some cause serious damage to crops and livestock, and some are even pathogenic in humans (Brusca & Brusca 1990). Wherever nematodes are found, they are the most abundant metazoan both as individuals and numbers of species. In 1934, Filipjev counted 4600 nematodes species, among them were 2,200 species free-living (nearly 1,000 continental and 1,200 marine) and 2,400 parasitic in animals (230 in invertebrates and 1,170 in vertebrates). Later, Andrássy (1992) estimated the numbers of free-living nematodes to 12,000–13,000 species. According to Hugot and colleagues (2001) 11,860 animal parasitic species were known, again doubling the total numbers of the known valid species of free-living and parasitic nematode to 24,000–25,000 species. In respect to the numbers of known species, Nematoda are thus the fourth greatest phylum after Arthropoda (1,105,000), MollIusca (50,000) and Vertebrata (44,000) (Andrássy 2005). Andrássy (1976) estimated the total numbers of species to 500,000, suggesting that merely 5% of the extant nematode species have been described to date. Characterizing nematode diversity is a true scientific challenge.

1.1.2 Nematodes as key organisms of the soil food web

Nematodes are an important component of the soil microfauna due to their abundance and diversity (Bongers & Ferris 1999). It is suggested that free-living nematodes play an important role in the decomposition pathways of soil food webs, and significantly contribute to nutrient cycling (de Ruiter *et al.* 1998). Moreover, these nematodes interact, directly or indirectly, with a variety of soil organisms, from bacteria (Traunspurger *et al*. 1997; de Mesel *et al*. 2004; Moens *et al*. 2005), fungi and earthworms to arthropods and molluscas. Since trophic groups of nematodes can be distinguished by their feeding apparatus, nematodes have been used to characterize functional changes in soil food webs and soil function (Ferris *et al.* 2001). Bacterivores often have fast reproduction cycles (eg. 2-3 weeks in *Caenorhabditis elegans*) (Marchant & Nicholas 1974), while predators and omnivores reproduce slowly (eg. 3-4 months in *Labronema ferox*) and therefore they are sensitive to disturbances (Korthals *et al*. 1996; Tenuta & Ferris 2004).

Nematode diversity studies historically focused on temperate regions and in 1998 only ten of 134 published ecosystem surveys were conducted in tropical forests (Boag & Yeates 1998). Almost 20 years later, soil nematode assemblages of tropical forests are still largely unexplored. Earlier studies suggested a peak of nematode diversity in temperate regions (Boag & Yeates 1998), recent studies have shown that tropical nematode communities can be extremely species rich. For example, Bloemer *et al.* (1997) investigating from 24 sites of a tropical forest in Mbalmayo, Cameroon found 431 nematode species belonged to 194 genera. Very recently, Porazinska *et al*. (2010, 2012) found for tropical forest in South America that total species richness was 300% higher in tropical than in temperate forests and more than 90% of the identified species belonged to new forms. It is now suggested that the low diversities reported earlier could be artefacts due to an inappropriate soil sampling strategy in the tropics (Lawton *et al*. 1996; Powers *et al*. 2009). Although nematode diversity is know from many study sites in the world, little attention has been paid to the question of how local nematode diversity depends on regional species richness and how land use intensity management affects the species richness and composition of trophic groups. Because tropical rainforests on different continents are characterized by vastly different abiotic and biotic conditions and evolved rather independently, we are not only lacking basic knowledge on the species inventory, but more so on the regional drivers of diversity and community assembly in these tropical regions (Corlett & Primack 2006; Kim & Byrne 2006; Grainger 2010).

Due to their small size and low mobility, their ubiquity, high diversity and high numbers, nematodes are ideally suited to investigate the drivers of community composition and food web functioning. According to distinct morphological differences in their feeding apparatus, nematodes are placed in five distinct functional groups (Yeates *et al*. 1993; Yeates & Bongers 1999): bacterivores, fungivores, herbivores, omnivores and predators (Yeates *et al.* 1993). Bacterivorous nematodes have a buccal cavity with tubiform shape (Fig. 1a), while fungivorous nematodes have an apparatus with short needle-shaped stylet and a developed median bulb (Fig. 1b). Herbivorous nematodes have a buccal cavity with a strong stylet, distinct knobs and a weak median bulb; or they have buccal cavity with a long needle-shaped stylet or odontostyle and a flask-shaped pharynx (Fig. 1c). Predator nematodes have an opening barrel shaped with big teeth or with a wide odontostyle (Fig. 1 d). Omnivorous nematodes differ from the other trophic groups by a variable buccal cavity with either small teeth or a short narrow odontostyle (Fig. 1e)

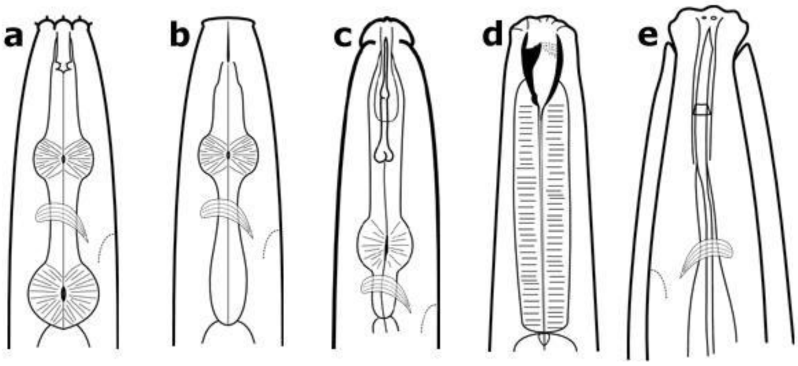

FIGURE 1. Nematodes can be classified into different feeding groups based on the structure of their mouthparts. a) bacterial feeder; b) fungal feeder; c) plant feeder; d) predator; and e) omnivore. (Zaborski 2014)

Accordingly, nematodes can be found at very different trophic positions in the soil food web (Fig. 2) where they rapidly respond to changing soil conditions. Several ecological indices have been proposed to characterise changes in the functional composition of nematodes in the soil food web (Bongers 1999; Wardle *et al*. 1995; Bongers & Ferris 1999; Mulder *et al*. 2009, 2011).

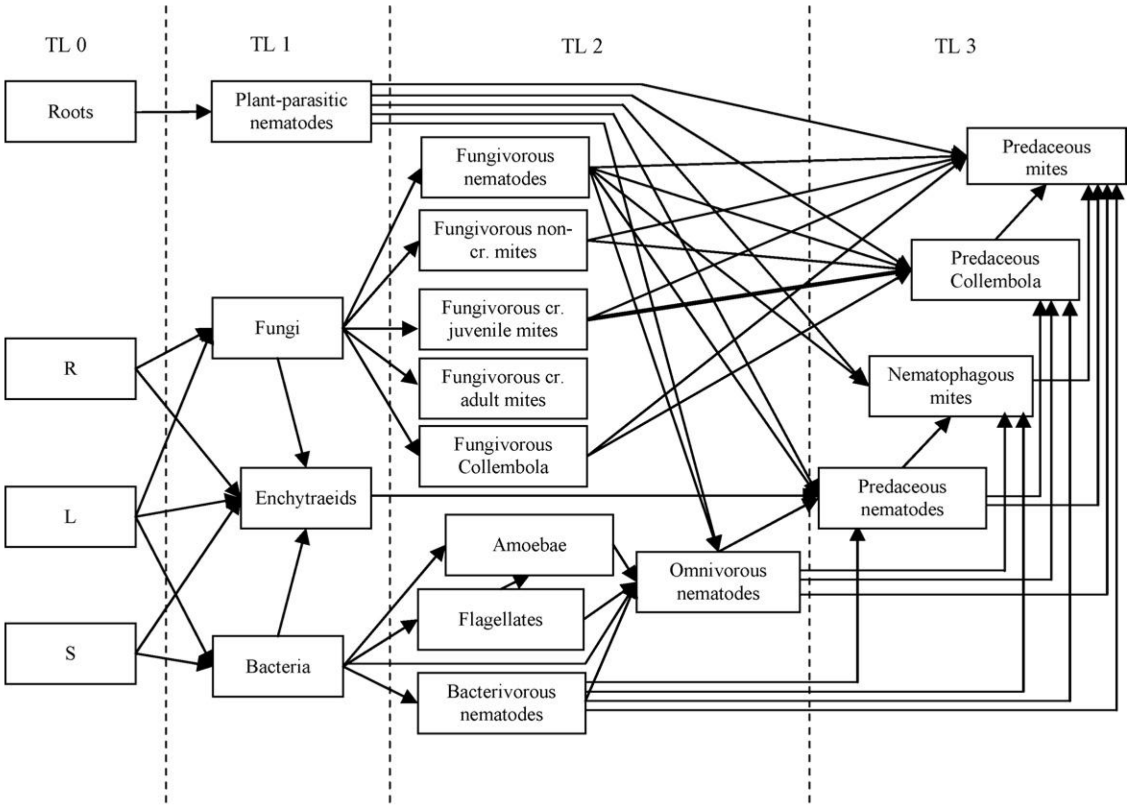

FIGURE 2. Schematic representation of different taxonomic and trophic groups in a soil food web. Arrows represent feeding links pointing to the consumer. TL = trophic level, R = recalcitrant organic matter, L = labile organic matter, S = water soluble sugars and cr = crypto stigmatic. (Holtkamp *et al.* 2008)

1.1.3 The nematode order Dorylaimida

The Dorylaimida (Pearse 1942) is a particularly diverse nematode order with many as yet unknown taxa in soil. With their long generation times 3-4 months (McSorley 2012) and production of few, but large eggs, dorylaimids can be classified as typical K-strategists, and are regarded as very sensitive to soil disturbances (Bongers *et al.* 1997). Being highly diverse and omnivorous, it has been proposed that they occupy key functions in microbial soil food webs (Zullini & Peretti 1986; Yeates *et al*. 1993; de Goede & Bongers 1994; Bongers & Bongers 1998).

Dorylaimida currently comprise 263 genera and 2637 recognized species (Andrássy 2009), but many new taxa are being described every year. The diversity of dorylaimids has been studied mostly in temperate regions, while their diversity and functional roles are poorly explored in the tropics. South East Asia, a part of the Oriental region (Cox 2001), is particularly understudied. Andrássy (1970) was the first author who recorded and described four species of dorylaimids in Vietnam, but since then, free-living dorylaimid forms have received little attention. During the last 10 years, several nematological surveys have been undertaken in the north of Vietnam to elucidate the diversity of this group.

From a morphological perspective, dorylaimids appear to be a well discriminable group, characterized by the combination of a series of apomorphic or autapomorphic features: the stoma is armed with a protruding structure, either a mural tooth or an axial odontostyle in a bottle-like pharynx. A pre-rectum is present at the hindermost portion of the intestine; and males possess a pair of ad-cloacal genital papillae, but caudal glands are absent. However, the current classification system, based on morphological data, is not satisfactory (Jairajpuri & Ahmad 1992; Peña-Santiago 2006; Vinciguerra 2006; Andrássy 2009), since the available molecular trees (e.g. Holterman *et al*. 2008) neither supports the traditional (morphological) subdivision in super families and families nor do they as yet provide alternative models. Therefore, further studies are urgently needed to clarify their evolutionary relationships in Dorylaimids, to uncover their true biodiversity, and to investigate their ecological functions.

1.1.4 A brief historical outline of nematological studies in Vietnam

The free-living dorylaimid nematodes have been little studied in Vietnam in comparison with plant parasitic nematodes. Andrássy (1970) was the first nematologist who described and recorded four species of dorylaims in Vietnam, namely *Mesodorylaimus orientalis* Andrássy*, Drepanodorylaimus brevicaudatus* Andrássy, *Actinolaimoides angolensis* (Andrássy) and *Discolaimoides filiformis* Das, Khan & Loof; most nematodes were collected from soil samples in 1966 in the northern regions of Vietnam. Later, Andrássy (1988, 2007) described two further species from the southern part of Vietnam: *Crocodorylaimus dimorphus* Andrássy and *Opisthodorylaimus cavalcantii* (Lordello). More recently, Ahmad & Sturhan (2000) described *Dorylaimellus vietnamensis* from Thanh Hoa Province, northern Vietnam, and Gagarin & Nguyen (2003, 2004, 2005) reported several new species from freshwater bodies in the Red River and Nhue River in North Vietnam: *Dorylaimus parvus* Gagarin & Nguyen*, Dorylaimellus vietnamicus* Gagarin & Nguyen, *Laimydorus oxurus* Gagarin & Nguyen and *Mesodorylaimus lutosus* Gagarin & Nguyen. Gagarin & Nguyen (2008a, b) recorded *D. parvus*, *L. oxurus*, *Mesodorylaimus lopadusae* Vinciguerra & La Fauci, *M. lutosus*, *Crocodorylaimus flavomaculatus* (von Linstow) and *Aquatides thornei* (Schneider). In a monographic contribution on free-living nematodes of Vietnam, Nguyen (2007) recorded eight genera and 17 species of dorylaims for Vietnam. Later, Vu *et al*. (2010) described two new species, namely *Crassolabium aenigmaticum* and *C. vietnamense*, in Nghe An and Ninh

Thuan provinces, respectively. Recently, Nguyen (2011) counted in total 26 species, 15 genera and eight families of dorylaims from Vietnam.

1.2 The Vietnamese landscape

1.2.1 Geography and climate

The mainland area of Vietnam is about 325,000 km² with mountains and hills covering three quarters of the country (Averyanov *et al.* 2003). The highest peaks of northern part in Vietnam are Fan Si Pan (3,143 m), Ta Giang (3,096 m), Si Lung (3,076 m). Vietnam is divided into eight geographical areas: the Northeast Region, the Red River Delta, the North Central Region, the South Central Region, the Central Highlands, the Southeast Region, and the Mekong Delta. Granite, an ancient metamorphous parent rock material and highly eroded karst ecosystems on limestone parent rock material are typical and widely distributed in the northeastern region of Vietnam (Averyanov *et al.* 2003; Sterling *et al.* 2006). Limestone karst formations, which are found at altitudes from 0 to over 2,000 m a.s.l. account for almost 20% of the country's surface area (Do, 2001). Averyanov *et al*. (2003) considered the Red River as the boundary between the western and eastern regions of northern Vietnam. Northeastern Vietnam contains both karst and granite formations: Ngan Son and Bac Son are two large karst formations, stand at 1,000 m a.s.l.; Viet Bac, Tam Dao, and Yen Tu Massifs are the largest granite formations, which lie on the edge of the delta. Other example of karst formations at lower altitude is Cat Ba Island. The island is the center of a now mostly submerged massif, which is visible only as scattered emergent hilltops of various heights. At intermediate altitude, the Lang Son karst formations are normally at elevations of 300–700 m. Nearer to the border with China, in the Cao Bang Provinces (Averyanov *et al.* 2003) they often reach over 1,600 m. The karst systems provide a variety of distinct microhabitats and are noted for their high levels of endemism.

The north-eastern region is characterized by a monsoon tropical climate with cold winter and summer rains (Averyanov *et al.* 2003). This coldness and seasonality is caused by the northeast monsoon winds that bring cold air from the edge of the Tibetan Plateau into northern Vietnam in the winter (from November to March) (Sterling *et al.* 2006). The hot weather and rain in summer result from the arrival of south-western monsoon winds blowing in from southerly oceans. Average annual temperature in the region are from 18^oC to 23.5^oC, average annual rainfall varies considerably from 1,300 mm (in Cho Ra, Bac Kan

Province) to 2,700 mm (in Huu Lung, Lang Son Province), and the average annual humidity ranges between 81–84%.

TABLE 1. Climate stations in northeastern Vietnam

Name	Elevation (m a.s.l.)	Temperature (^oC)		Rainfall (mm)		Average annual humidity (%)
		Annual average	Range monthly average	Annual average	Range monthly average	
Cao Bang	>1000	22	14-27	1440	16–267	81
Lang Son	258	21	13–27	1400	23–255	82
Cat Ba	12	23	16-28	1800	20-330	85

http://www.weatherbase.com/weather/weather.php3?s=62884

1.2.2 Biodiversity

Vietnam's biodiversity has been investigated by scientists for several reasons: the country harbors a globally significant diversity of species; scientists have recently described an unexpectedly large number of new species and a high proportion of its species are endemic (Sterling *et al.* 2006). Vietnam is situated in the Indo-Burma ecological region, which is known as one of the biodiversity hotspots of the world. It borders with the contiguous territories of Laos, Thailand, Cambodia and China making up the eastern region of Indochinese Peninsula, which hosts a great variety of vegetation types, rich plant communities and a wide variety of habitats. The southern tip of Vietnam meets the Gulf of Thailand; Laos and Cambodia bound the country to the west and the East Sea lies to the east (Sterling *et al.* 2006). Botanists estimate that 13,000 vascular plant species live in Vietnam, yet fewer than 10,000 have been recorded so far.

1.2.3 Land use intensity (anthropogenic impact)

A major reason of concern for below-ground diversity is the impact of anthropogenic forcing on tropical systems (Brook *et al.* 2008; Tylianakis *et al.* 2008). Deforestation and conversion of forest land for agriculture has increased dramatically in Southeast Asia since the 1980's (Hansen & DeFries 2004). The current deforestation rates recorded in this region are the highest worldwide (Achard 2002; Shearman *et al.* 2012). In Vietnam, data from the Forest Resources Assessment (FAO 1993) reported the highest rates of deforestation during the last decade, and illegal logging destroyed large regions of forest during 1990s. Deforestation was considered as one of the main elements of the development crisis in Vietnam's mountain areas, becoming a serious social-economic problem (Jamieson *et al.* 1998). Local case studies have shown that large-scale agricultural areas increased at the expense of forest cover in many regions in the country (D'haeze *et al.* 2005).

Ecologists focused mainly on the aboveground "visible" part of tropical ecosystems, largely neglecting the enormous diversity and important functions of soil organisms (Beare *et al*. 1997; Swift *et al.* 1998; Wardle *et al.* 2004; Cock *et al.* 2012). Natural and planted forests, and primary and secondary forests, provide important ecosystem services (Perz 2007). Land use change from primary to secondary forest and to agriculture is known to result in drastic qualitative and quantitative changes to soil biodiversity, function and nutrient cycling (Giller *et al.* 1997). The major effects are due to clearing and burning of forest biomass, leading to decreases in soil biota, soil organic matter content and nutrients, which are further reinforced by conversion to cultivation (Lee & Pankhurst 1992).

1.3 Nematode community analysis

The last decade, numerous studies analyses nematode communities in soil environments. In Sweden, several ecological studies on nematodes developed (e.g. Sohlenius 1973), Russia (e.g. Tsalolikhin 1976), Poland (e.g. Wasilewska 1970), Italy (e.g. Zullini 1976), and in Germany (e.g. Sudhaus 1981). In the USA, Coleman and colleagues led the 'Man and the Biosphere' program at the National Resource Ecology Laboratory in Colorado Springs (e.g. Yeates & Coleman 1982), and Crossley and colleagues (e.g. Stinner & Crossley 1982) continued these studies at the Institute of Ecology of the University of Georgia. In the same time period, Yeates developed a very productive program on the ecology of soil nematodes in New Zealand (e.g. Yeates 1979). In the early 1980s, concerns regarding soil pollution and its impact on the functioning of soils were increasing.

The idea of using nematode communities and ecological indices as indicators of ecosystem quality (e.g. diversity, stability, and resilience) has received increased attention over the last decade (de Goede & Dekker 1993; Freckman & Ettema 1993; Verschoor *et al.* 2001). Indices may be useful tools because they not only provide quantitative means to characterize an ecosystem, but also to compare different ecosystems. Different ecological indices (i.e. the Maturity Index (MI), the Plant-Parasite Index (PPI), the Basal Index, Enrichment index, Structure index and Channel Index) have been developed in temperate ecosystems to describe shifts in soil functioning and ecosystem services based on changes in the composition of nematode trophic groups. To better understand the changes in the nematode fauna, de Goede *et al.* (1993) proposed a triangle which combined c-p groups 3-5 and plotting c-p = 1, c-p = 2 and c-p = (3-5). The triangles also provied very useful in mornitoring study.

Bongers *et al.* (1989) finally characterized nematode into five trophic groups along a r-K scale, and proposed the Maturity Index to evaluate the disturbance of the environment. Nematodes can be considered to be "colonizers" (r-strategists) that rapidly increase in number under favourable conditions. On the other hand, nematodes can be considered to be "persisters" (K-strategists) that have low colonization ability and are sensitive to disturbance (Bongers 1990; Bongers *et al*. 1991). Colonizer (c) and persisters (p) are assigned on scale (c-p) from 1-5 respectively. Nematodes of c–p 1 have a short life cycle, high fecundity, are tolerant to disturbance, and can be assigned to r-strategists. In contrast, nematodes of c–p 5 produce few large eggs, have a long life cycle and are sensitive to disturbance resembling K-strategists. The Maturity Index is calculated as the weighted mean of the individual c-p values: MI = $\sum_{i=1}^{n} v(i) * f(i)$.

The particular cp-value is represented by v(i), while f(i) is the frequency of a particular taxon (Bongers 1990). The low MI indicates for the disturbed of environment and the high MI show the stable of the habitat. The Plant Parasite Index is similar to the MI but calculated only for the plant-feeding nematodes. The MI2-5 index is similar to the MI except that MI2-5 excludes the c-p1 enrichment opportunistic species. The ΣMI2-5 index sums the MI values for all nematodes in the c-p2–5 range.

Ferris *et al.* 2001 proposed further an Enrichment Index (EI), Struture Index (SI) and Channel Index (CI) to characterize the food webs structure, enrichment, and decomposition channels. These indices are calculated from the weighted abundance of nematode guilds. Bacterivores (Bax), fungivores (Fux), predators (Cax), and omnivores (Omx) are considered as nematode guilds ranging along the c–p scale from x = 1 to x = 5. The nematode fauna is comprised of basal, enrichment and structural components (b,e,s)

$b=(Ba2+Fu2)*W_2$, where $W_2= 0.8$

$e=(Ba1*W_1)+(Fu2*W_2)$, where $W_1 = 3.2$ and $W_2= 0.8$

$s=(Ba_n*W_n+Ca_n*W_n+Fu_n*W_n+Om_n*W_n)$ where n=3-5, $W_3= 1.8$, $W_4= 3.2$, $W_5= 5.0$

($W_1 = 3.2$; $W_2= 0.8$; $W_3= 1.8$, $W_4= 3.2$, $W_5= 5.0$)

The Enrichment (EI), Structure (SI), Basal (BI), and Channel (CI) indices are calculated from the weighted faunal components (Ferris *et al.* 2001):

$EI = 100*e/(e+b)$

$SI = 100*s/(s+b)$

$BI = 100*b/(e+s+b)$

$CI = 100\ Fu2*W_2/(Ba1*W_1+Fu2*W_2)$.

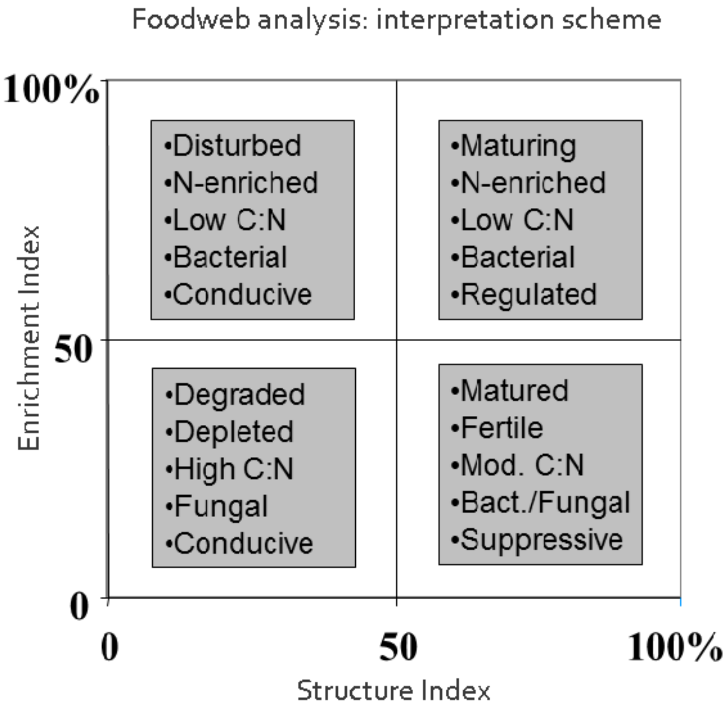

FIGURE 3. Interpretation scheme of the soil food web and its environment based on weighted nematode faunal analysis. Quadrats refer to faunal ordination in the faunal profile (Nematode INdicator Joint Analysis: https://sieriebriennikov.shinyapps.io/ninja/)

Accordingly, the EI describes whether a soil ecosystem is nutrient enriched (high EI) or depleted (low EI). The Structure Index represents an aggregation of functional guilds with cp values ranging from 3 to 5. The SI describes whether a soil ecosystem is structured/matured (high SI) or disturbed/degraded (low SI). Faunal analyses based on these indices have been shown to provide insights into changes in the food web structure and energy flows in soil systems (Ferris *et al.* 2001), and allow the deduction of testable hypotheses based on the relative enrichment and structure of the system (Fig. 3).

The Channel Index provides a means to estimate the relative flow of energy through the fungal and bacterial decomposition channels. Indices of fungal and bacterial activity based on the relative abundance of fungal- and bacterial-feeding nematodes have been proposed several times following the calculation of their relative proportions in grasslands, woodlands and cultivated fields (Twinn 1974). The CI differs from the indices above by including weighting parameters for the size and metabolic rates of the nematode indicators.

Aims

The nematode communities were compared in three spatially separated karst mountain systems: Pia Oac Nature Reserve (Cao Bang Province), Huu Lien Nature Reserve (Lang Son Province) in the mainland of north eastern Vietnam, and of Cat Ba National Park (Hai Phong Province) island in the Gulf of Tonkin. The major aim of this study was to investigate how local land-use intensification affects nematode diversity, community composition and their functional roles in the soil food web. For this a series of hypotheses arise:

1. The taxonomic and genetic diversity of free-living nematodes species in tropical forest has been vastly underestimated.
2. Land-use intensity (LUI) had strong impacts on nematode communities (i.e. functional group level).
3. Changes in nematode communities along gradients of LUI were driven by abiotic soil conditions.

2. Material and methods

2.1 Study areas

The study was conducted in three sites: Pia Oac Nature Reserve ($22^{0}32'$ – $22^{0}40'$N, $105^{0}49'$ – $105^{0}57'$E), Cao Bang Province; Huu Lien Nature Reserve ($21^{0}37'$ – $21^{0}45'$N, $106^{0}19'$ – $106^{0}29'$E), Lang Son Province and Cat Ba National Park ($20^{0}44'$ – $20^{0}51'$N, $106^{0}58'$ – $106^{0}45'$E) Hai Phong Province. The field sites were situated in the north-east of Vietnam, where the climate conditions correspond to monsoon tropical with a mean annual temperature of 25^{o}C with the lowest temperature in January and the highest in July (Tordoff *et al*. 2000). A mean of annual precipitation of approximately 1,433 mm and the relative ambient humidity of 81% are recorded (Sterling *et al*. 2006). Elevation ranges in Cao Bang, Lang Son and Cat Ba are 1,931m, 638 and around 100m, respectively. The sites used were selected based on the land use gradients in limestone karst areas of Cao Bang/Lang Son in the main land of northeastern Vietnam and on Cat Ba Island in the Gulf of Tonkin (Fig. 4). The topography of northeastern Vietnam is characterized by extensive karst features, visible in a variety of formations, such as steep towers, cone shaped mountains and hills, caves and depressed areas.

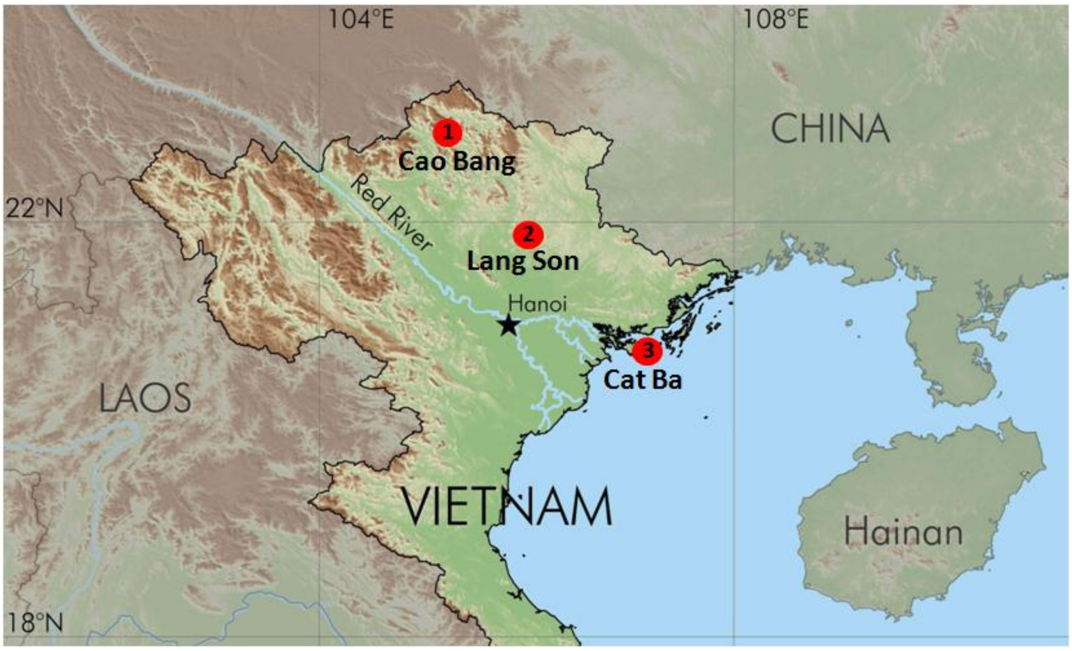

FIGURE 4. The study sites (marked with red dots) in the north-eastern part of Vietnam: 1) Cao Bang; 2) Lang Son; and 3) Cat Ba. (Map was provided by Kevin Koy).

Vegetation of study areas

The North-east of Vietnam belongs to the South-Chinese floristic unit, which also includes the south-eastern areas of Yunnan, south and south-western areas of Guangxi, the coastal area of Nanning to Macao, the Luichow Peninsula, and the island of Hainan. The flora in this region has developed on very specific soil and environmental conditions from ancient, highly eroded karst ranges, supporting a unique flora and vegetation. Tropical evergreen and semi-deciduous, broad –leaved and mixed forest spread across areas of limestone rocky outcrops mainly at elevations from the sea level up to 1,600 m a.s.l., although the main part of these regions occur between 300–900 m a.s.l. The deciduous broad-leaved or coniferous forests also occur along the highest ridges, sometimes between 1,400–1,700 m (Averyanov *et al.* 2003). The primary vegetation consists of dominant species of: *Burretiodendron hsienmu, Gleditchia* sp., *Symingtonia populnea.* Lower subcanopy and undergrowth strata in these forests are well developed and composed of numerous tree and shrub species from some of the families such as: Annonaceae (*Mitrephora*), Clusiaceae (*Calophyllum*), Ebenaceae (*Diospyros*), Euphorbiaceae (*Trigonostemon*), Moraceae (*Dimerocarpus, Ficus, Streblus, Teonongia*), Oleaceae (*Jasminum*), Rosaceae (*Eriobotyta*), Rubiaceae (*Canthium*), Rutaceae (*Murraya*), Sapindaceae (*Xerospermum*), Sapotaceae (*Sinosideroxylon*), Sterculiaceae (*Heritiera, Pterospermum*) (Nguyen 1994, 1997a). The dominant species in these floristically rich forests are subendemic coniferous trees: *Pinus kwangtungensis*, *Pseudotsuga brevifolia*, and *Tsuga chinensis* (Nguyen 1998). Other rare gymnosperm species like *Amenthotaxus argotaenia*, *A. hatuyensis*, *A. yunnanensis*; *Cupressus torulosa*, *Taxus chinensis*, and *Xanthocyparis vietnamensis* are also more or less regular components of the limestone forests within the Vietnamese part of the South-Chinese province (Averyanov *et al.* 2003).

2.2 Soil sampling

Three sites (Site 1, Cao Bang; Site 2, Lang Son; and Site 3, Cat Ba) were selected for soil sampling in May 2013 (Fig. 4). Each site has four land-use types (Fig. 5): i) largely undisturbed primary forest, ii) disturbed secondary forest, iii) cassava fields characteristic of slash and burn, and iv) agriculture intensively managed agricultural fields (maize, rice). In each land-use type, six bulk soil samples were taken. Each bulk soil sample was pooled by mixing 5 soil cores in a crosswise grid design in a 10 m² plot. After removal of the litter layer, the soil samples were collected from 0-10 cm depth with a 3.5 cm diameter soil corer. In total, 24 composite soil samples were collected in each site as independent replicates. Each soil was mixed and passed through a sieve with 2mm mesh size to remove small stones and roots. A part of the soil was used for measurements of chemical properties (soil moisture, total C, N) and the other part was used for nematode extraction. The nematodes were extracted directly from the field. Nematodes were fixed in 4% formaldehyde for the morphological analysis, and in DESS (Yoder 2006) for molecular phylogeny reconstruction.

2.3 Soil analysis

The C and N content and the carbon-nitrogen ratio (C/N) of the soil samples were measured. The soil was dried and grinded with a vibration mill (MM 400, Retsch). 30 mg weight was measured with a NC Soil Analyzer (Flash 2000, Thermo Scientific) following the instructions by Thermo Scientific, USA.

2.4 Extraction, processing and mounting of nematodes

Nematodes were extracted from 100 fresh g of soil samples by extraction technique (Bearmann, 1917) and Flegg’s (1967) sieving method. The extracted nematodes were relaxed, killed by hot water (70^{o}C), and fixed in 4% formaldehyde. Total nematode numbers were counted under stereomicroscope (x50 magnification) and 200 individuals were picked out randomly for indentification. The fixed individuals were gradually transfered to anhydrous glycerol following De Grisse’s (1969) technique, and mounted on permanent glass slides. The nematodes were identified to genus level by using the keys from Andrássy, 2009, Ahmad & Jairajpuri, 2010.

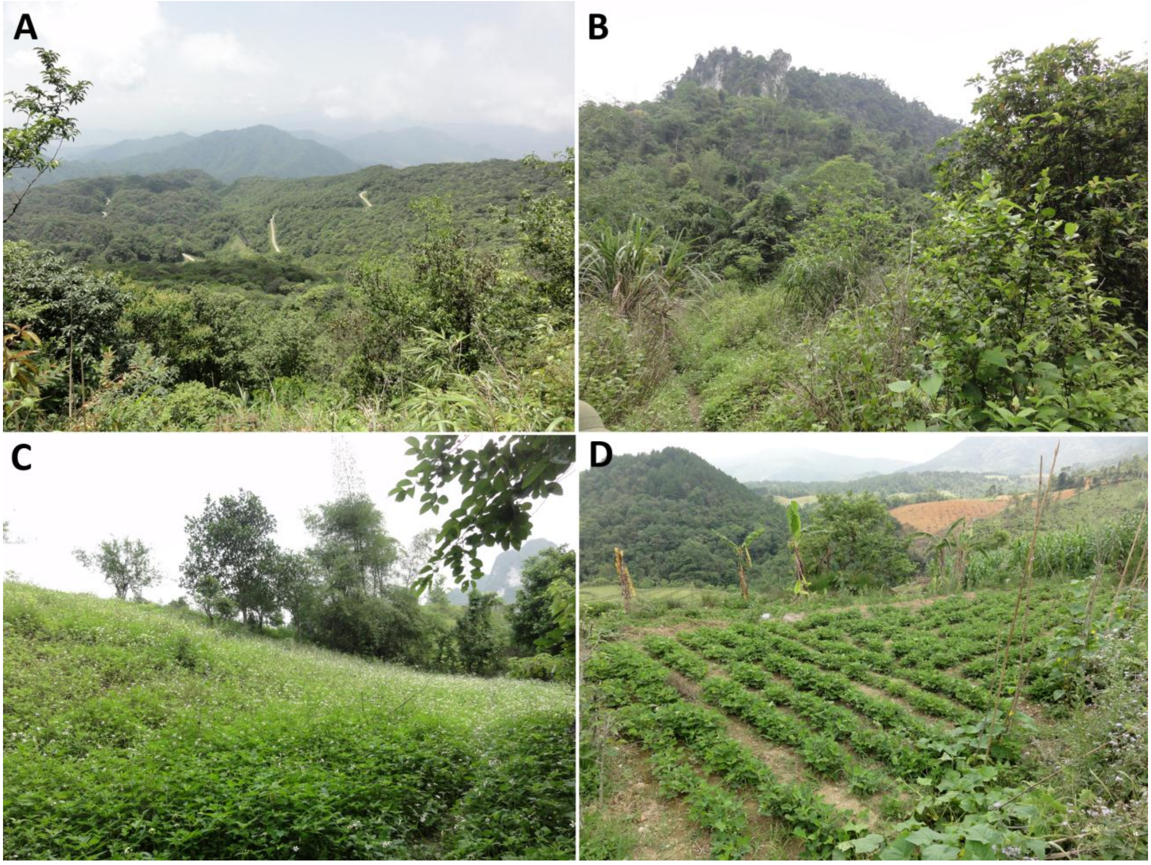

FIGURE 5. Four different land use intensity habitats in Cao Bang: A. Primary forest; B. Secondary forest; C. Slash and burn; and D. Intensive agriculture.

2.5 Morphological characterization of nematodes: LM and SEM

Light microscopy

The nematodes were identified to genus level by using several identification keys (Andrássy 2009, Ahmad & Jairajpuri 2010). Functional diversity of nematode assemblages was assigned using individual trophic levels according to Yeates *et al.* 1993: 1) bacterivorous nematode (Ba), 2) fungivorous nematodes (Fu), 3) herbivorous nematodes (Plant Parasitic Nematode) (PPN), 4) omnivorous nematodes (Om), and 5) predator nematodes (Pr). Taxonomic groups were also assigned to colonizer-persistors c-p values, according to Bongers (1990)**.** Measurements of specimens were taken using a light Olympus BH-2 microscope equipped with differential interference contrast (DIC). Morphometrics included de Man's indices and most of the usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof & Coomans (1970) and spicule terminology follows Peña-Santiago *et al.* (2014). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe® Photoshop® CS. Drawings were made using a camera lucida.

Scanning electron microscopy

After their examination and identification, a few specimens preserved in glycerine were selected for observation under SEM following the protocol by Abolafia & Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin SEM microscope.

2.6 Molecular analysis of nematodes

DNA was extracted from single individuals using the proteinase K and Worm Lysis Buffer protocol (William *et al.* 1992). Nematode was transferred to an 0.5 mL Eppendorf tube containing 18 µl of Worm Lysis Buffer (WLB) (50 mM KCL, 10mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40, and 0.45% Tween 20) and 2 µL proteinase K (600 µg ml^{-1}) (Thermo Scientific). The tubes were incubated at 65^{o}C (1h) and then at 95^{o}C (15 min). The PCR was performed in a 30 µL final volume containing 24,9 µL of sterile water, 0.6 µL of each PCR primer, 0.6 µL dNTP, 0.3 µL Taq-polyerase, 3 µL Buffer 10x Thermo Scientific Green and 1 µL of DNA extracted solution. The PCR amplification profile consisted of for min at 94^{o}C, 35 cycles of 1 min at 94 ^{o}C, 1.5 min at 55^{o}C and 2 min at 72^{o}C, followed by a final step of 10 min at 72^{o}C. The primer used for amplification were D2A (5´-ACAAGTACCGTGAGGGAAAGTTA-3´) and D3B (5´-TCCTCGGAAGGAACCAGCTACTA-3´) for amplification of D2-D3 region of 28S (Subbotin *et al.* 2006).

The PCR products were purified using Exo-SAP PCR cleanup containing 7 µL of PCR product, 0.15 µl Exonuclease I (Exo), 0.9 µl Shrimps Phosphatase Alkali (SAP) and 1.95 µl sterile water were incubated on 37°C for 20 minutes and then heated up to 85°C for 15 minutes, and then the purified solution was diluted ten times. The sequencing reaction was performed with 1 µL of DNA purify template, 0.25 µL BigDye v3.1, 2.25 µL 5x BigDye sequencing buffer, 0.25 µL of one primer. The mixture was heated up for 10 s at 96°C, 5s at 55°C repeated for 32 cycles followed by 4 min at 60°C. The sequencing was performed at the Cologne Center for Genomics (CCG). The sequences obtained were submitted to the GenBank.

The newly obtained sequences were aligned with other forty six D2-D3 expansion segments of 28S rDNA gene sequences available in GenBank using ClustalX 1.83 (Thompson *et al.*

1997). Outgroup taxa were chosen according to the results of previously published reviews (Holterman *et al*. 2008; Álvarez-Ortega *et al*. 2013a). Sequence alignments were manually edited using GenDoc 2.6.002 (Nicholas *et al*. 1997). The sequence dataset was analysed with Bayesian inference (BI) and Maximum Likelihood (ML) using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and MEGA 6 (Tamura *et al*. 2013), respectively. The best fit model of DNA evolution for BI was obtained using the program MrModeltest 2.2 (Nylander 2004) with the Akaike Information Criterion in conjunction with PAUP* 4b10 (Swofford 2003). BI analysis under the GTR+G+I model was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 10^6 generations. ML analysis was implemented under the best-fitting evolutionary model (GTR+I+G), obtained using the program MEGA 6, and 1000 bootstrap replications. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. The trees were visualized with the program FigTree v1.4.0 and drawn with Adobe Acrobat XI Pro 11.0.1.

2.7 Statistical analysis

Statistical analyses were performed in R, version 3.2.2. The community data on the genus level (and on the functional group level) based on Bray-Curtis similarity was analyzed to assess the differences between the location and land-use intensity (LUI). Non-metric multidimensional scaling (NMDS) was used to visualize the results. Analysis of Variance (ANOVA) and subsequent Tukey Tests were used to analyze the effects of regions and LUI on nematode taxa and trophic groups. Differences at the $P < 0.05$ level were considered to be statistically significant. Additionally, a Permutation Multivariate Analysis of Variance (PERMANOVA), Discriminant Function Analyses (DFAs) and Pearson product-moment correlation (Pearson-correlation) were applied for the data.

Soil nematodes indices such as MI, PPI, CI and Enrichment index calculated NINJA website (Nematode INdicator Joint Analysis: https://sieriebriennikov.shinyapps.io/ninja/)

3. Publications

Publication 1

New data of three rare belondirid species (Nematoda, Dorylaimida, Belondiridae) from Vietnam, with the first record and description of the male of *Oxybelondira paraperplexa* Ahmad & Jairajpuri, 1979

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***Biodiversity Data Journal*, 2: e1156. DOI: 10.3897/BDJ.2.e1156.**

Biodiversity Data Journal 2: e1156
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Taxonomic paper

New data of three rare belondirid species (Nematoda, Dorylaimida, Belondiridae) from Vietnam, with the first record and description of the male of *Oxybelondira paraperplexa* Ahmad & Jairajpuri, 1979

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Abstract

Three rare nematode species of the family Belondiridae, originally described from and only known to occur in India are recorded for the first time in Vietnam: *Axonchium thoubalicum*, *Belondira murtazai* and *Oxybelondira paraperplexa*. It is the first report of these three genera in this country. The three species are described, including new morphological data, morphometrics and light microscope pictures. The male of *O. paraperplexa* is collected and described for the first time. It is characterized by its 1.54 mm long body, ad-cloacal pair of genital papillae situated at 9.0 μm from the cloacal aperture, only one ventromedian supplement located at 15 μm from the ad-cloacal pair within the range of spicules, spicules slightly curved ventrad and 42 μm long (7 times as long as wide and 2 times as long as

cloacal body diameter), and tail 100 µm long (c = 15, c' = 5) and similar to that of the female.

Keywords

Description, nematodes, new records, Oriental region, taxonomy

Introduction

Dorylaims, the representatives of the nematode order Dorylaimida, with more than 2500 valid species and more than 250 valid genera (Andrássy 2009), are one of the most important taxa among Nematoda. Their diversity has been characterized with some success in several temperate (Europe, New Zealand, South Africa and USA) and a few tropical (Costa Rica and India) areas, but it remains poorly explored or nearly totally unknown in many other territories. The study of dorylaimid fauna of southeast Asia, and more particularly of Vietnam, has received little attention as only 25 species belonging to 15 genera were identified in this country until the end of the past decade in a total of 12 contributions (see Table 1 for a summary of available data). More recent studies by Nguyen (2011), however, suggest that the Vietnamese dorylaimid fauna is significantly richer.

Table 1.

Previous records of dorylaims in Vietnam.

Species	References
Actinolaimoides angolensis (Andrássy, 1963) Siddiqi, 1982	Andrássy (1970)
Aporcelaimellus krygeri Heyns, 1965	Nguyen (2007)
A. obtusicaudatus (Bastian, 1865) Heyns, 1965	Nguyen (2007)
Aquatides thornei (Schneider, 1937) Heyns, 1968	Gagarin and Nguyen (2008a)
Crassolabium aenigmaticum Vu, Abolafia, Ciobanu & Peña-Santiago, 2010	Vu et al. (2010)
C. vietnamense Vu, Abolafia, Ciobanu & Peña-Santiago, 2010	Vu et al. (2010)
Crocodorylaimus dimorphus Andrássy, 1988	Andrássy (1988), Nguyen (2007)
C. flavomaculatus (von Linstow, 1876) Andrássy, 1988	Nguyen (2007), Gagarin and Nguyen (2008b)
Discolaimoides filiformis Das, Khan & Loof, 1969	Andrássy (1970)
Dorylaimellus vietnamensis Ahmad & Sturhan, 2000	Ahmad and Sturhan (2000)
D. vietnamicus Gagarin & Nguyen, 2004	Gagarin and Nguyen (2004), Nguyen (2007)
Dorylaimoides micoletzkyi (de Man, 1921) Thorne & Swanger, 1936	Nguyen (2007)
Dorylaimus parvus Gagarin & Nguyen, 2003	Gagarin and Nguyen (2003), Gagarin and Nguyen (2008b)
D. stagnalis Dujardin, 1845	Nguyen (2007)
Drepanodorylaimus brevicaudatus Andrássy, 1970	Andrássy (1970)

Labronema neopacificum Rahman, Jairajpuri, Ahmad & Ahmad, 1986	Álvarez-Ortega et al. (2010)
Laimydorus oxurus Gagarin & Nguyen, 2005	Gagarin and Nguyen (2005), Nguyen (2007)
L. pseudostagnalis (Micoletzky, 1927) Siddiqi, 1969	Nguyen (2007)
Mesodorylaimus derni Loof, 1969	Nguyen (2007)
M. lopadusae Vinciguerra & La Fauci, 1978	Nguyen (2007), Gagarin and Nguyen (2008b)
M. lutosus Gagarin & Nguyen, 2005	Gagarin and Nguyen (2005), Nguyen (2007)
M. mesonyctius (Kreis, 1930) Andrássy, 1959	Nguyen (2007)
M. orientalis Andrássy, 1970	Andrássy (1970)
Opisthodorylaimus cavalcantii (Lordello, 1955) Carbonell & Coomans, 1986	Andrássy (2007)
Prodorylaimus longicaudatoides Altherr, 1968	Nguyen (2007)

The information regarding the occurrence of members of the family Belondiridae Thorne, 1939 in Vietnam is especially poor as it is limited to the original description of two species of the genus *Dorylaimellus* Cobb, 1913, namely *D. vietnamensis* Ahmad & Sturhan, 2000 and *D. vietnamicus* Gagarin & Nguyen, 2004. This contribution provides new data on three known belondirid genera and species, which are recorded for the first time in the country.

Materials and methods

Nematological surveys were conducted in three locations of Northern Vietnam: Cuc Phuong National Park, Ninh Binh Province, in August 2009; Phong Nha Ke Bang National Park, Quang Binh Province, in July 2010; Huu Lien Nature Reserve, Lang Son Province, in May 2013. Soil samples from each location consisted of 200 g of soil from up to 10 cm depth. Soil samples were kept in plastic bags and brought to laboratory. Nematodes were extracted by a modified Baermann funnel technique, killed by heat, fixed in hot formaldehyde 4%, transferred to anhydrous glycerol according to Siddiqi (1964), and mounted on glass slides for further handling.

Microphotographs were taken with a Nikon Eclipse 80i light microscope provided with differential interference contrast optics (DIC) and a Nikon Digital Sight DS-U1 camera. Specimens were deposited in the collections of the Institute of Ecology and Biological Resources (IEBR), Vietnam; the Andalusian Research Group on Nematology, University of Jaén, Spain and the Institute for Zoology, Department of Terrestrial Ecology, University of Cologne, Germany.

Taxon treatments

Axonchium thoubalicum Dhanachand & Jairajpuri, 1981

Material

a. country: Cuc Phuong National Park, Vietnam; stateProvince: Ninh Binh; verbatimLocality: in soil around roots of *Parashorea chinensis*, karst forest; verbatimElevation: 300-400m;

verbatimLatitude: 20°19’00” N; verbatimLongitude: 105°36’30” E; decimalLatitude: 20.316666; decimalLongitude: 105.6083333; eventDate: August, 2009; individualCount: 4; sex: 0 male, 4 females; recordedBy: Nguyen T. A. D; collectionID: Cuc Phuong 4.1 (7); Cuc Phuong 4.1 (16); institutionCode: IEBR; collectionCode: Nematode

Description

Specimens examined (n=4): Four females in good condition (Figs 1, 2).

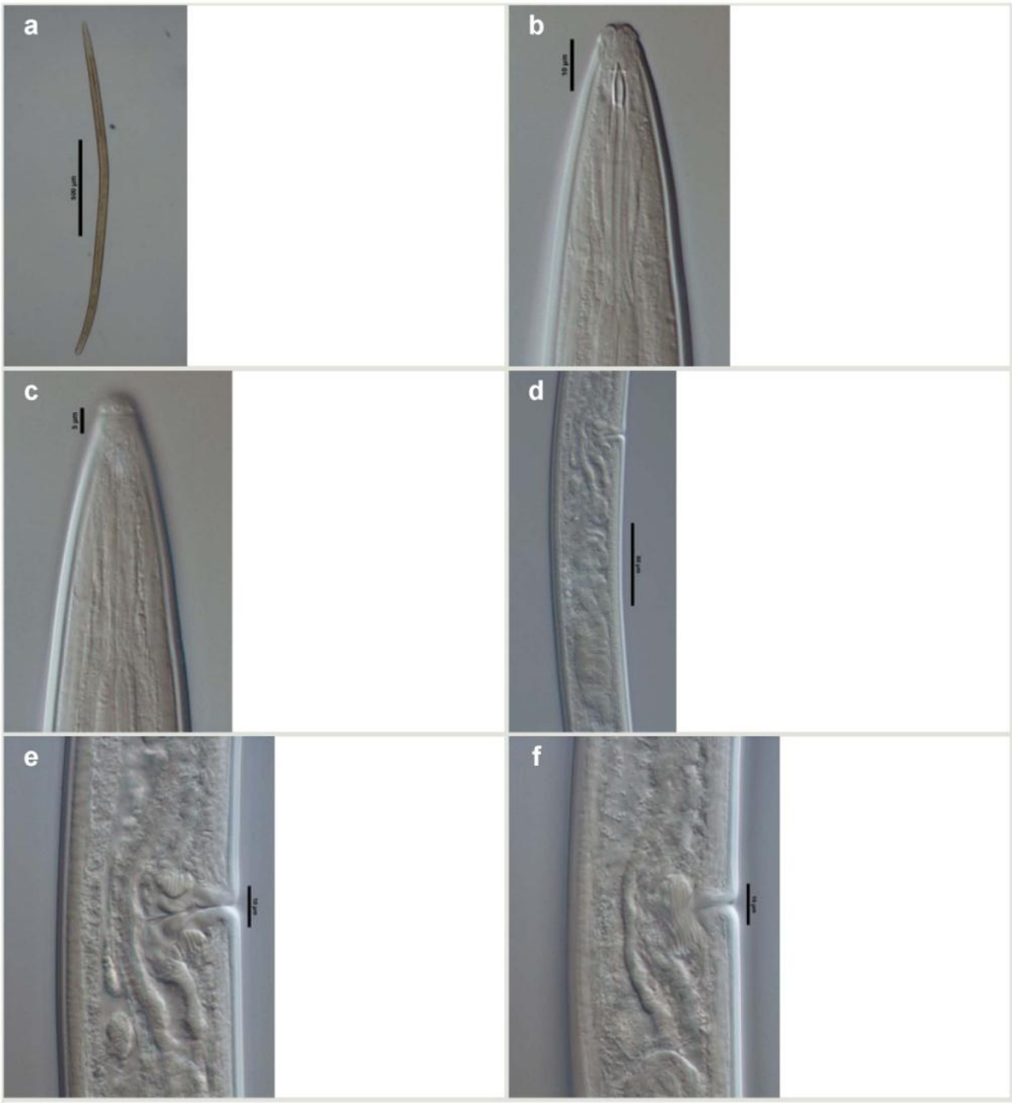


Figure 1.

Axonchium thoubalicum Dhanachand & Jairajpuri, 1981 (Female, LM)

a: Entire
b: Anterior region in median view
c: Lip region in submedian view
d: Posterior genital branch
e: Vagina
f: Vagina

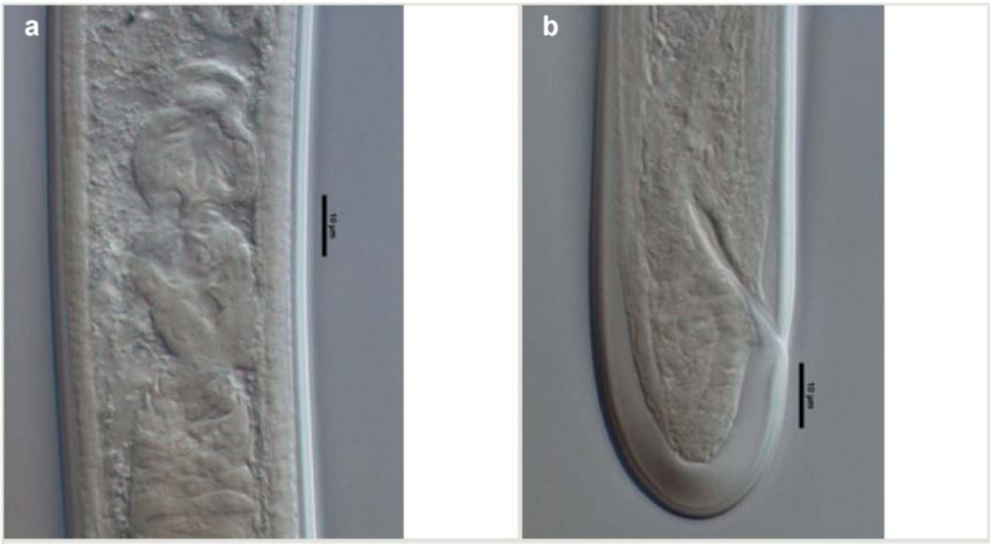


Figure 2.

Axonchium thoubalicum Dhanachand & Jairajpuri, 1981 (Female, LM)

a: Oviduct-uterus junction

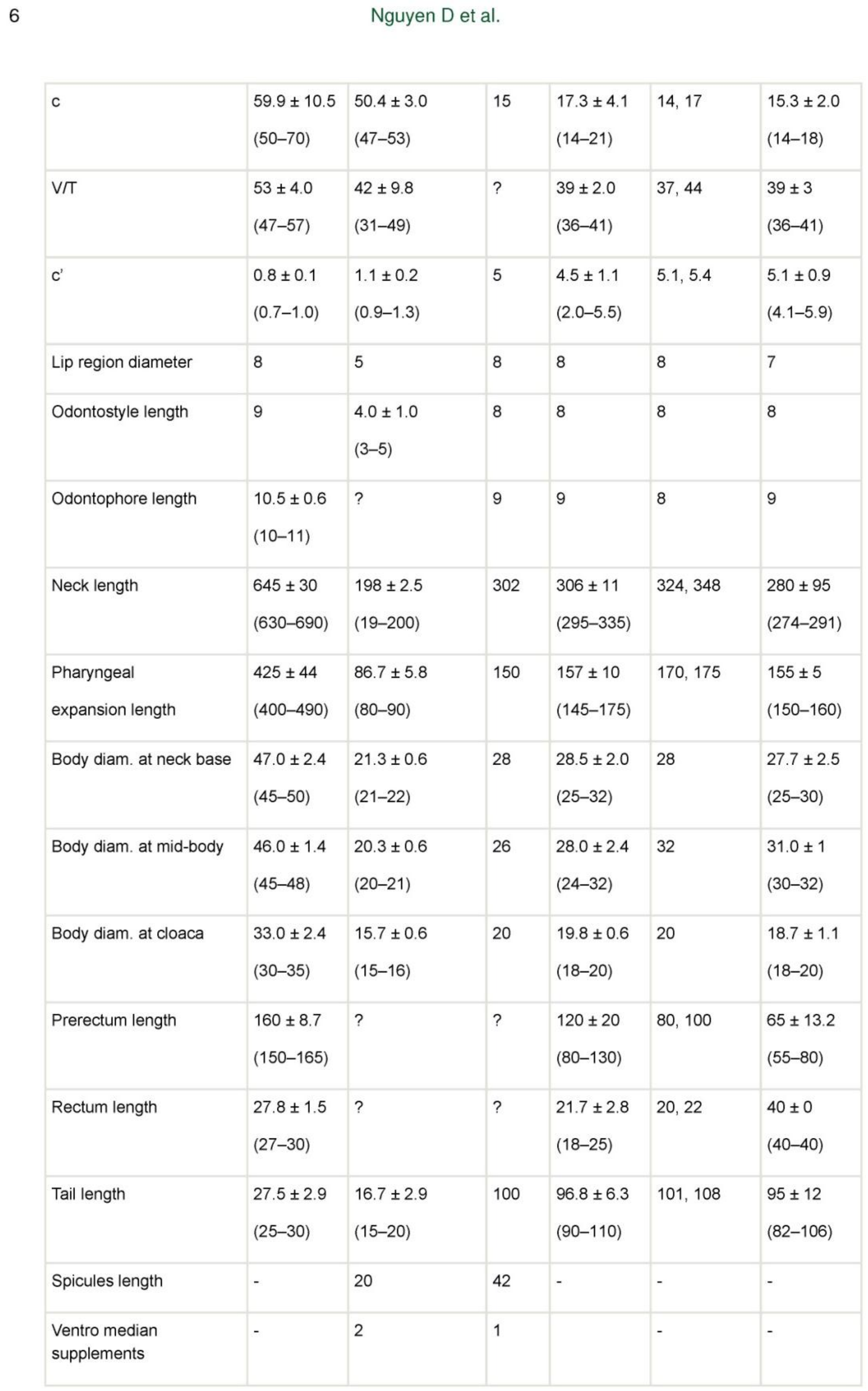
b: Caudal region

Measurements: See Table 2.

Table 2.

Morphometrics of *Axonchium thoubalicum* Dhanachand & Jairajpuri, 1981, *Belondira murtazai* Siddiqi, 1968 and *Oxybelondira paraperplexa* Ahmad & Jairajpuri, 1979 from Vietnam. All measurements in µm except L in mm.

Species	***A. thoubalicum***		***O. paraperplexa***			
Natural Reserve	**Cuc Phuong**		**Cuc Phuong**		**Phong Nha**	**Huu Lien**
Province	**Ninh Binh**		**Ninh Binh**		**Quang Binh**	**Lang Son**
n	**4**♀♀	**3**♂♂	♂	**12**♀♀	**2**♀♀	**6**♀♀
Character						
L	1.63 ± 0.12 (1.50–1.75)	0.83 ± 0.09 (0.77–0.94)	1.54	1.55 ± 0.07 (1.46–1.68)	1.51, 1.77	1.44 ± 0.12 (1.43–1.45)
a	35.3 ± 1.7 (33–37)	41.1 ± 5.2 (38–47)	59.4	55.6 ± 3.9 (50–62)	44, 52	46 ± 1.7 (45–48)
b	2.5 ± 0.3 (2.2–2.8)	4.2 ± 0.5 (3.9–4.8)	5.1	5.1 ± 0.1 (4.9–5.4)	4.7, 5.1	5.2 ± 0.2 (4.9–5.3)



c	59.9 ± 10.5 (50–70)	50.4 ± 3.0 (47–53)	15	17.3 ± 4.1 (14–21)	14, 17	15.3 ± 2.0 (14–18)
V/T	53 ± 4.0 (47–57)	42 ± 9.8 (31–49)	?	39 ± 2.0 (36–41)	37, 44	39 ± 3 (36–41)
c'	0.8 ± 0.1 (0.7–1.0)	1.1 ± 0.2 (0.9–1.3)	5	4.5 ± 1.1 (2.0–5.5)	5.1, 5.4	5.1 ± 0.9 (4.1–5.9)
Lip region diameter	8	5	8	8	8	7
Odontostyle length	9	4.0 ± 1.0 (3–5)	8	8	8	8
Odontophore length	10.5 ± 0.6 (10–11)	?	9	9	8	9
Neck length	645 ± 30 (630–690)	198 ± 2.5 (19–200)	302	306 ± 11 (295–335)	324, 348	280 ± 95 (274–291)
Pharyngeal expansion length	425 ± 44 (400–490)	86.7 ± 5.8 (80–90)	150	157 ± 10 (145–175)	170, 175	155 ± 5 (150–160)
Body diam. at neck base	47.0 ± 2.4 (45–50)	21.3 ± 0.6 (21–22)	28	28.5 ± 2.0 (25–32)	28	27.7 ± 2.5 (25–30)
Body diam. at mid-body	46.0 ± 1.4 (45–48)	20.3 ± 0.6 (20–21)	26	28.0 ± 2.4 (24–32)	32	31.0 ± 1 (30–32)
Body diam. at cloaca	33.0 ± 2.4 (30–35)	15.7 ± 0.6 (15–16)	20	19.8 ± 0.6 (18–20)	20	18.7 ± 1.1 (18–20)
Prerectum length	160 ± 8.7 (150–165)	?	?	120 ± 20 (80–130)	80, 100	65 ± 13.2 (55–80)
Rectum length	27.8 ± 1.5 (27–30)	?	?	21.7 ± 2.8 (18–25)	20, 22	40 ± 0 (40–40)
Tail length	27.5 ± 2.9 (25–30)	16.7 ± 2.9 (15–20)	100	96.8 ± 6.3 (90–110)	101, 108	95 ± 12 (82–106)
Spicules length	-	20	42	-	-	-
Ventro median supplements	-	2	1		-	-

Female: Slender nematodes of medium size. Habitus very weakly curved ventrad upon fixation. Body cylindrical, tapering towards both ends, but more so towards the anterior one. Cuticle bearing fine transverse striations, about 2.0 µm thick at neck region, 2.0 µm at mid-body, and 8–10 µm at tail. Lateral chords 7–8 µm wide or occupying one-fifth of mid-body diameter. Lip region cap-like, offset from adjacent body by a constriction, twice as wide as high and less than one-fifth (16–18%) of body diameter at neck base; lips separate, their inner portion forming liplets; papillae low, hardly protruding. Amphid fovea cup-shaped, its opening at level of the cephalic constriction and occupying 6 µm or *ca* three-fourths of lip region diameter. Odontostyle fusiform, as long as lip region diameter, with aperture occupying one-third of its total length. Guiding ring simple but distinct, located at 9 µm or 1.1 times the lip region diameter from the anterior end. Odontophore simple, rod-like. Pharynxbipartite, consisting of a slender muscular anterior section, which bears a minute (but perceptible) mucro at its beginning (observed in the four specimens examined); a deep constriction separating both sections; basal expansion nearly cylindrical, occupying 63–71% of total neck length and surrounded by a distinct spiral muscular sheath. Cardia conoid to cylindroid. Genital system mono-opisthodelphic, with the anterior branch reduced to an uterine sac *ca* twice the body diameter long whereas the posterior one is well developed: reflexed ovary does not reach the oviduct-uterus junction, oocytes first in two rows and then apparently in a single row; oviduct joining the ovary sub-terminally and consisting of a slender portion with prismatic cells and a moderately developed *pars dilatata* with distinct lumen; conspicuous sphincter between oviduct and uterus; uterus long, tripartite, consisting of a proximal wider region, narrower and longer intermediate section and a nearly sphaerical distal part; vagina 20–23 µm long, extending inwards *ca* one-half of body diameter, with *pars proximalis* surrounded by a very perceptible sphincter, *pars refringens* totally absent and *pars distalis* well developed; vulva a transverse slit. Prerectum long, 4.7–5.2 anal body widths long. Rectum shorter, 0.8–0.9 times anal body width. Tail short and rounded.

Male: Not found.

Distribution

Axonchium thoubalicum Dhanachand and Jairajpuri 1981 was collected in Cuc Phuong National Park, in soil around roots of *Parashorea chinensis* in karst forest.

Taxon discussion

This species is known to occur onlyin India, from where it was originally described on the basis of three females and one male, and later reported by Gambhir and Dhanachand (1990), who provided measurements of two females and two males. The Vietnamese material herein examined perfectly fits the general morphology of the type material (females) (unfortunately, male was not collected in Vietnam), especially concerning the genital system. Moreover, the morphometrics of the two Indian populations and the Vietnamese one largely overlap in spite of the few number of available specimens in the three cases. The ranges of several ratios and measurements, however, are significantly widened, for instance *c* = 50–70 *vs* 53–55 in type material, *V* = 47–57 *vs* 52–54, etc. Thus, no reasonable uncertainty persists on the identity of this material.

Notes

This is the first record of this genus and this species in Vietnam, which might display a Oriental biogeographical range.

Belondira murtazai Siddiqi, 1968

Nomenclature

syn. *B. rafiqi* Suryawanshi 1972, by Ferris et al. (1983)

Material

a. country: Cuc Phuong National Park, Vietnam; stateProvince: Ninh Binh; verbatimLocality: in soil around roots of *Parashorea chinensis* in karst forest.; verbatimElevation: 300-500m; verbatimLatitude: 20°19’28’’ N; verbatimLongitude: 105°39’30’’ E; decimalLatitude: 20.3244444; decimalLongitude: 105.6583333; eventDate: August, 2009; individualCount: 4; sex: 3 males, 1 female; recordedBy: Nguyen T. A. D; collectionID: Cuc Phuong 1.1 (38); Cuc Phuong 4.3 (23); institutionCode: IEBR; collectionCode: Nematode

Description

Specimens examined (n=4): One female and three males in good condition (Fig. 3).

Mesurements: See Table 2.

Adult: Slender to very slender nematodes of small size. Habitus upon fixation nearly straight in female and slightly curved ventrad in males, especially in posterior body region. Body cylindrical, tapering towards both ends, but more so towards the anterior extremity. Cuticle thin, bearing fine transverse striations throughout the body. Lateral chords 4 µm wide, occupying ca one-fifth (20%) of mid-body diameter. Lip region continuous, tapering, somewhat truncate, 1.7 times as wide as high and *ca* one-fourth (25%) of body diameter at neck base; labial framework weakly sclerotized; lips amalgamated, with low papillae. Amphid fovea difficult to observe in the specimens examined. Odontostyle very short and narrow, but having perceptible lumen and aperture. Guiding ring simple. Pharynx consisting of a slender and weakly muscular anterior region which enlarges rather abruptly, pharyngeal expansion nearly cylindrical, occupying about one-half of total neck length and surrounded by a weak but well distinguishable spiral muscular sheath. Cardia rounded conoid, enveloped by the intestinal wall.

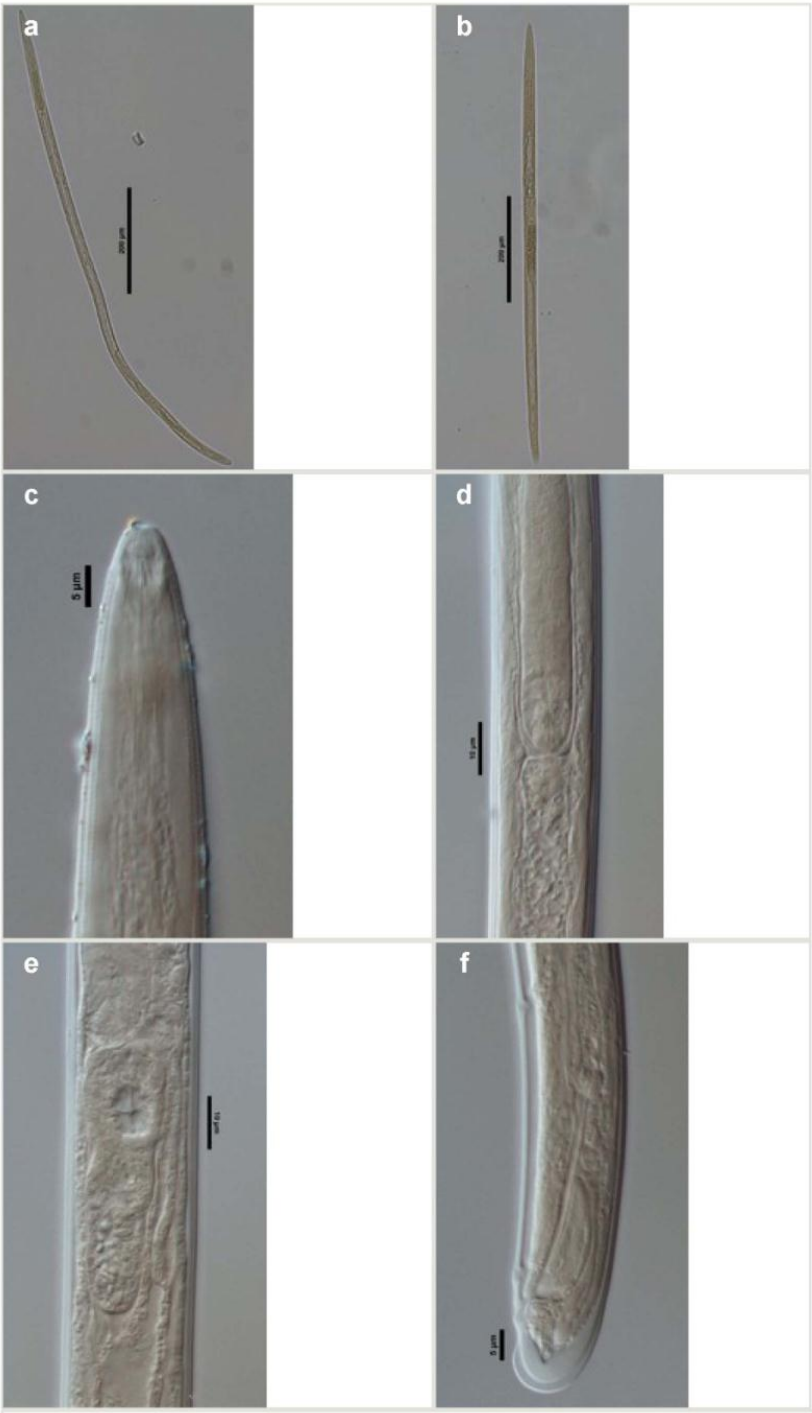


Figure 3.

Belondira murtazai Siddiqi, 1968 (LM)

a: Male entire
b: Female entire
c: Male anterior region
d: Male pharyngo-intestinal junction
e: Vagina and anterior uterine sac (ventral view)
f: Male posterior caudal region and spicules

Female: Genital system mono-opisthodelphic. Anterior branch rudimentary, reduced to a uterine sac up to 1.5 times the corresponding body diameter long. Posterior branch well developed, but the detailed composition of its tract indistinguishable in the only one specimen examined. Tail rounded, slightly clavate, with the outer cuticle layer visibly thickened and showing radial striation.

Male: Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 5 μm from cloacal aperture, there are two ventromedian supplements, the posteriormost of which is located out of the range of the spicules, at 45 μm from the ad-cloacal pair. Spicules dorylaimoid, slightly curved ventral, 6.3 times as long as wide and 1.2 times as long as anal body diameter. Lateral guiding pieces difficult to observe. Tail short and rounded, visibly concaveventrally, the outer cuticle layer less expanded than in the female. Caudal pores, if present, obscure.

Distribution

Belondira murtazai Siddiqi 1968 was collected in Cuc Phuong National Park, in soil around roots of *P. chinensis* in karst forest.

Taxon discussion

Above description fits very well the original one of this species by Siddiqi (1968) and the revised one by Ferris et al. (1983), the latter based on the study of type material. A few minor differences, however, may be noted in the morphometrics of Indian and Vietnamese populations, but their ranges widely overlap, for instance slightly smaller general size (L = 0.77–0.94 *vs* 0.85–1.06 mm in type material as described by Siddiqi) and somewhat longer odontostyle (3–5 *vs* 3–4 μm). A major tentative difference between both populations is the length of the prevulval uterine sac (up to 1.5 *vs* 2.3–3.0 times the body diameter); nevertheless, the morphometrics given by Siddiqi certainly covers only a few out of the 12 female paratypes as Ferris et al., who examined two female paratypes loaned by Siddiqi, stated (p. 26) that the "anterior uterine branch is 1.7–2.0 body widths long", and their Fig. 11E shows that this structure is hardly more than 1.5 times the body diameter. Ferris et al. (*op. cit.*) regarded *B. rafiqi* Suryawanshi, 1972, also recorded in India, as a junior synonym of *B. murtazai*, a decision that seems to be well supported and is herein followed.

Notes

This is the first record of this genus and this species in Vietnam, which might display a Oriental biogeographical range.

Oxybelondira paraperplexa Ahmad & Jairajpuri, 1979

Material

a. country: Vietnam; stateProvince: Cuc Phuong National Park in Ninh Binh, Huu Lien Nature Reserve in Lang Son, Phong Nha – Ke Bang National Park in Quang Binh; verbatimLocality: Soil samples of karst forest; verbatimElevation: 300-500m; eventDate: Ninh Binh: in August, 2009; Lang Son: in May, 2013; Quang Binh: in July, 2010; individualCount: 21; sex: 1 male, 20 females; recordedBy: Nguyen T.A.D; collectionID: Cuc Phuong 5.1 (10); Cuc Phuong 1.1 (20); Cuc Phuong 3.2 (11); Cuc Phuong 3.2 (15); Huu Lien 15.1; PN-KB 27.1; institutionCode: IEBR; collectionCode: Nematode

Description

Specimens examined (n=21): Twenty females and one male in good condition (Figs 4, 5).

Mesurements: See Table 2.

Adult: Very slender nematodes of medium size. Habitus slightly curved ventrad after fixation. Body cylindrical, gradually tapering towards both extremities, but more so towards the posterior end. Cuticle thin, with fine transverse striations. Lateral chords 9–12 µm wide, occupying ca one-third of mid-body diameter. Lip region continuous, somewhat truncate, 1.7–2.0 times as wide as high and *ca* one-fifth (20%) of body diameter at neck base; labial framework well developed, having distinct labial and post-labial sclerotizations; lips amalgamated, with low papillae. Amphid fovea cup-shaped. Cheilostom a truncate cone, lacking any differentiation. Odontostyle rather strong, 1.2–1.4 times the lip region width long, with distinct lumen and aperture, which occupies *ca* one-fourth of its length. Guiding ring simple, located at 7 µm or one lip region diameter from the anterior end. Odontophore rod-like, 1.3 times the odontostyle length. Pharynx consisting of a slender part that enlarges gradually, and the basal expansion nearly cylindrical, occupying 50–56% of total neck length and surrounded by a distinct spiral muscular sheath. Cardia rounded conoid, as long as wide.

Female: Genital system mono-opisthodelphic. Anterior branch nearly lacking or reduced to a very short, vestigial sac. Posterior branch well developed: ovary 72–90 µm long, reaching and occasionally surpassing the oviduct-uterus junction, with oocytes first in several rows and then apparently in one row; oviduct joining the ovary subterminally and consisting of a slender portion with prismatic cells and moderately developed *pars dilatata* with distinct lumen; a marked sphincter separates oviduct and uterus; uterus 60–70 µm long or 2.0–2.5 times the corresponding body diameter. Vagina 13–16 µm long or extending inwards *ca* one-half of body diameter: *pars proximalis* as long as wide, with somewhat sigmoid walls and enveloped by weak circular musculature; *pars refringens* lacking; *pars distalis* well developed. Vulva a pre-equatorial, transverse slit. Prerectum 6.3–6.5 anal body widths long. Rectum as long as one anal body width. Tail elongate, made of two sections of about equal length: the anterior onewider, and tapering gradually the posterior narrower and cylindrical, visibly clavate at the end; hyaline portion one-third to two-fifths of tail length.

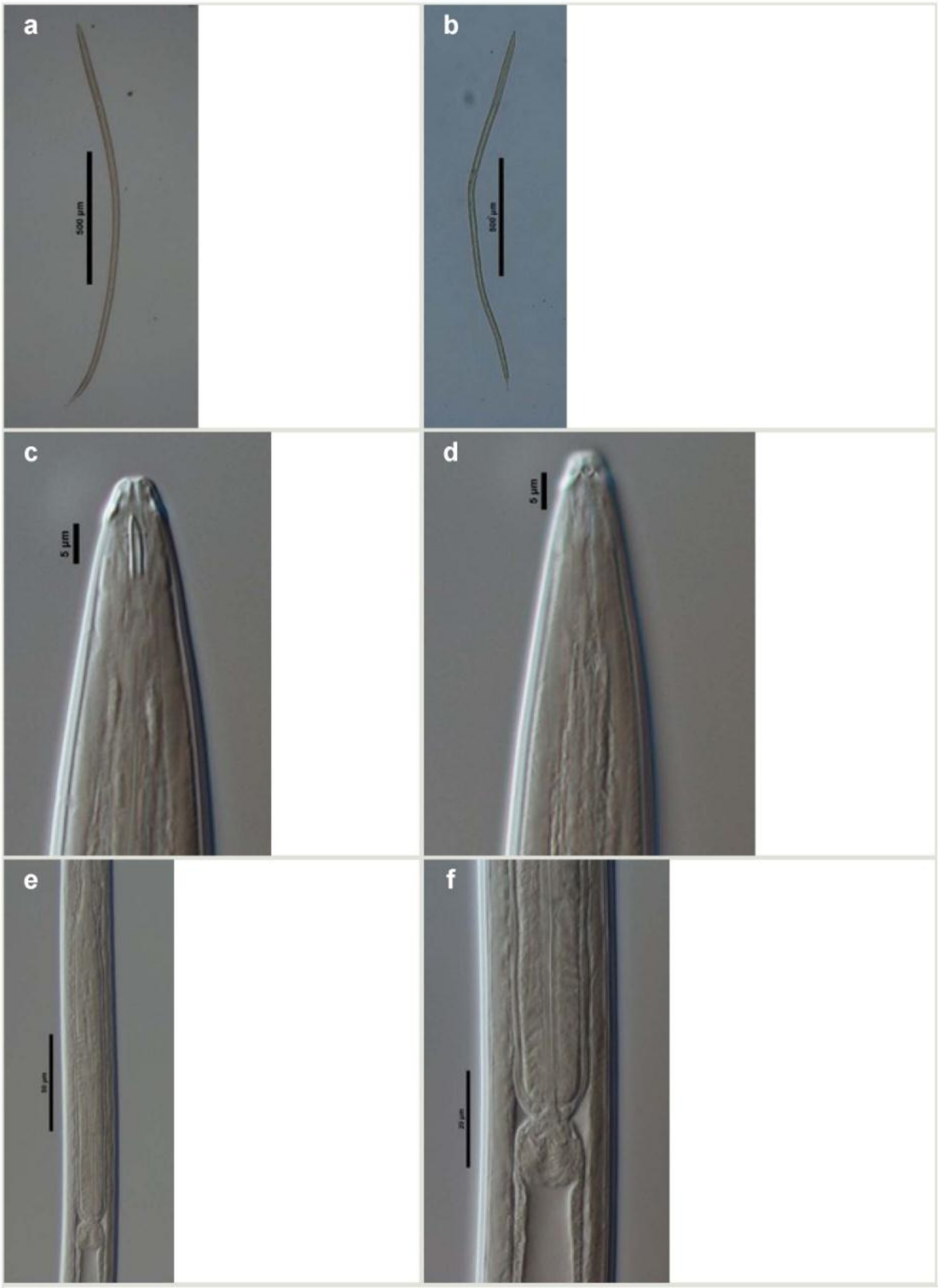


Figure 4.

Oxybelondira paraperplexa Ahmad & Jairajpuri, 1979 (LM)

a: Male entire
b: Female entire
c: Female anterior region in median view
d: Female anterior region in surface lateral view
e: Female enlarged section of the pharynx
f: Female pharyngo-intestine junction

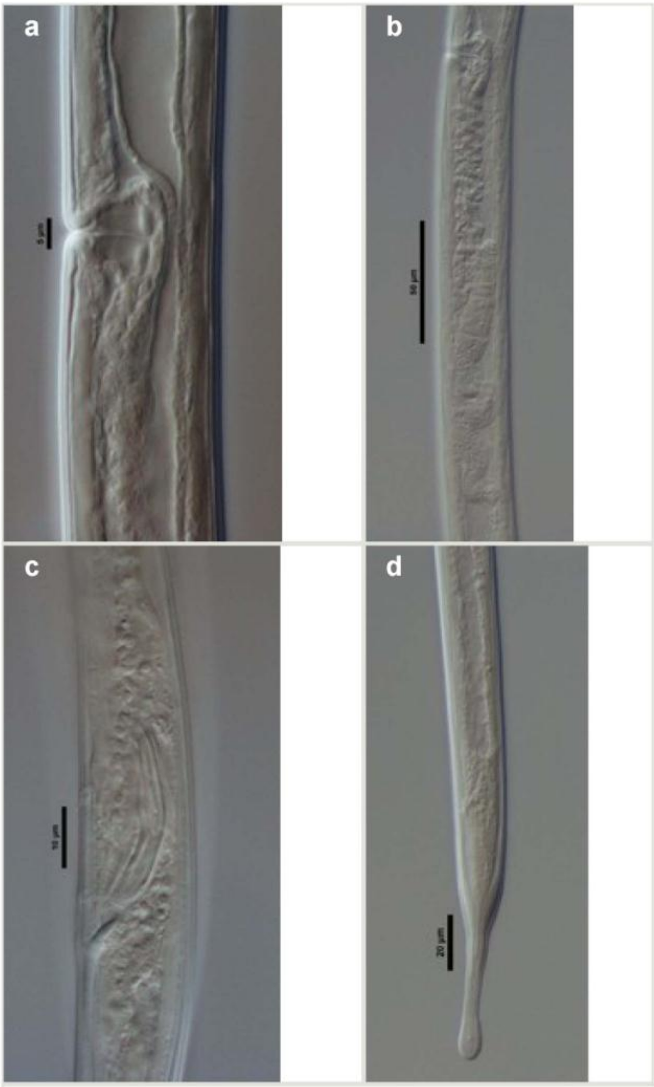


Figure 5.

Oxybelondira paraperplexa Ahmad & Jairajpuri, 1979 (LM)

a: Female vagina
b: Female genital system
c: Male spicules
d: Female posterior body region

Male: Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 9 µm from the cloacal aperture, one ventromedian supplement within the range of spicules, located at 15 µm from ad-cloacal pair. Spicules dorylaimoid, slightly curved ventrad and relatively slender, 7 times as long as wide and 2 times as long as anal body diameter. Lateral guiding pieces not well seen. Tail elongate, made of two sections of about equal length:anterior one wider and tapering gradually at both sides, posterior narrower and cylindrical, visibly clavate at the end; hyaline portion one-third to two-fifths of tail length.

Distribution

Oxybelondira paraperplexa was collected in Cuc Phuong National Park and Huu Lien Nature Reserve in North Vietnam, and Phong Nha – Ke Bang National Park in central Vietnam, collected in soil samples from karst forests.

Taxon discussion

This is the first record of *O. paraperplexa* after its original description from Manipur, India by Ahmad and Jairajpuri (1979), the the male is described for the first time. The Vietnamese females are identical to the type material, but new morphological data are herein provided and the ranges of the morphometrics appreciably widened.

Acknowledgements

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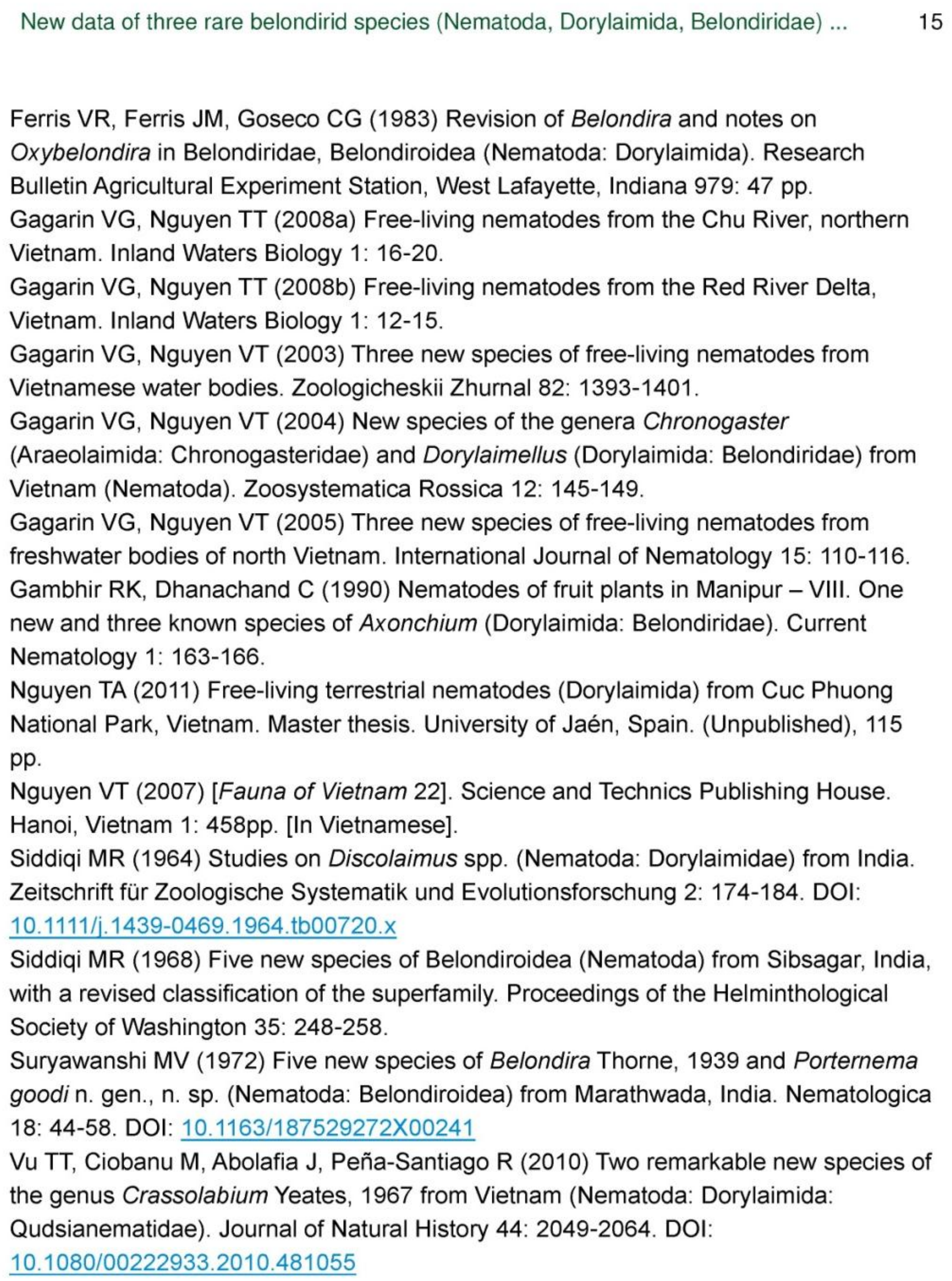
References

- Ahmad M, Jairajpuri MS (1979) *Oxybelondira* n. gen. (Dorylaimida: Oxydiridae) with description of two new species. Indian Journal of Nematology 8: 25-31.
- Ahmad W, Sturhan D (2000) Description of five new species of Dorylaimida (Nematoda). International Journal of Nematology 10: 55-66.
- Álvarez-Ortega S, Vu TT, Peña-Santiago R (2010) Studies on four species of the genus *Labronema* Thorne, 1939 (Dorylaimida, Qudsianematidae). Journal of Nematode Morphology and Systematics 13: 107-122.
- Andrássy I (1970) Freilebende Nematoden aus Vietnam. Opuscula Zoologica Budapestinensis 10: 5-31.
- Andrássy I (1988) The superfamily Dorylaimoidea (Nematoda) – a review. The family Dorylaimidae. Opuscula Zoologica Budapestinensis 23: 3-63.
- Andrássy I (2007) Contribution to the genus *Opisthodorylaimus* Ahmad &Jairajpuri, 1982 (Nematoda: Dorylaimida), with description of two new species. Opuscula Zoologica Budapestinensis 36: 3-17.
- Andrássy I (2009) Free-living nematodes of Hungary. III. Hungarian Natural History Museum, Budapest, 608 pp. [ISBN 978-963-508-574-3]
- Dhanachand CH, Jairajpuri MS (1981) Description of male *Axonchium amplicolle* and of two new species of *Axonchium* from Manipur, India. Nematologica 27: 95-102. DOI: 10.1163/187529281X00098



- Ferris VR, Ferris JM, Goseco CG (1983) Revision of *Belondira* and notes on *Oxybelondira* in Belondiridae, Belondiroidea (Nematoda: Dorylaimida). Research Bulletin Agricultural Experiment Station, West Lafayette, Indiana 979: 47 pp.
- Gagarin VG, Nguyen TT (2008a) Free-living nematodes from the Chu River, northern Vietnam. Inland Waters Biology 1: 16-20.
- Gagarin VG, Nguyen TT (2008b) Free-living nematodes from the Red River Delta, Vietnam. Inland Waters Biology 1: 12-15.
- Gagarin VG, Nguyen VT (2003) Three new species of free-living nematodes from Vietnamese water bodies. Zoologicheskii Zhurnal 82: 1393-1401.
- Gagarin VG, Nguyen VT (2004) New species of the genera *Chronogaster* (Araeolaimida: Chronogasteridae) and *Dorylaimellus* (Dorylaimida: Belondiridae) from Vietnam (Nematoda). Zoosystematica Rossica 12: 145-149.
- Gagarin VG, Nguyen VT (2005) Three new species of free-living nematodes from freshwater bodies of north Vietnam. International Journal of Nematology 15: 110-116.
- Gambhir RK, Dhanachand C (1990) Nematodes of fruit plants in Manipur – VIII. One new and three known species of *Axonchium* (Dorylaimida: Belondiridae). Current Nematology 1: 163-166.
- Nguyen TA (2011) Free-living terrestrial nematodes (Dorylaimida) from Cuc Phuong National Park, Vietnam. Master thesis. University of Jaén, Spain. (Unpublished), 115 pp.
- Nguyen VT (2007) [*Fauna of Vietnam* 22]. Science and Technics Publishing House. Hanoi, Vietnam 1: 458pp. [In Vietnamese].
- Siddiqi MR (1964) Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. Zeitschrift für Zoologische Systematik und Evolutionsforschung 2: 174-184. DOI: 10.1111/j.1439-0469.1964.tb00720.x
- Siddiqi MR (1968) Five new species of Belondiroidea (Nematoda) from Sibsagar, India, with a revised classification of the superfamily. Proceedings of the Helminthological Society of Washington 35: 248-258.
- Suryawanshi MV (1972) Five new species of *Belondira* Thorne, 1939 and *Porternema goodi* n. gen., n. sp. (Nematoda: Belondiroidea) from Marathwada, India. Nematologica 18: 44-58. DOI: 10.1163/187529272X00241
- Vu TT, Ciobanu M, Abolafia J, Peña-Santiago R (2010) Two remarkable new species of the genus *Crassolabium* Yeates, 1967 from Vietnam (Nematoda: Dorylaimida: Qudsianematidae). Journal of Natural History 44: 2049-2064. DOI: 10.1080/00222933.2010.481055

Publication 2

Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), with an updated taxonomy of the genus

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Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), with an updated taxonomy of the genus

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Abstract

Three new species of *Aporcelaimoides* from natural habitats in Vietnam are studied, described and illustrated, including line drawings, LM and/or SEM pictures. *Aporcelaimoides brevistylum* **sp. n.** is characterized by its body 1.95–2.90 mm long, lip region offset by deep constriction and 17–18 µm broad, ventral side of mural odontostyle 11–14 µm long with aperture occupying 62–71% of its length, neck 663–767 µm long, pharyngeal expansion occupying 58–66% of total neck length, uterus a simple tube 85–182 µm long, *pars refringens vaginae* absent, V = 55–63, tail short and rounded (34–46 µm, c = 49–76, c' = 0.6–0.8), spicules 67–86 µm long, and one ventromedian supplement out the range of spicules. *Aporcelaimoides minor* **sp. n.** is distinguished in having body 2.09–2.61 mm long, lip region offset by deep constriction and 19–20 µm broad, mural odontostyle 14–16 µm long at its ventral side with aperture occupying 73–84% of its length, neck 579–649 µm long, pharyngeal expansion occupying 57–66% of total neck length, uterus a simple tube 44–69 µm long, *pars refringens vaginae* well developed, V = 48–56, female tail very short, rounded conoid or truncate (14–26 µm, c = 90–146, c' = 0.3–0.6), and male unknown. *Aporcelaimoides silvaticum* **sp. n.** is characterized by its body 2.09–2.60 mm long, lip region offset by depression and 17–18 µm broad, mural odontostyle 11–12 µm long at its ventral side with aperture occupying 60–66% of its length, neck 597–720 µm long, pharyngeal expansion occupying 58–64% of total neck length, uterus a simple tube 128–243 µm long, *pars refringens vaginae* well developed, V = 58–60, tail short and rounded (27–37 μm, c = 67–94, c' = 0.6–0.7), spicules 64–75 μm long, and two or three widely spaced ventromedian supplements bearing hiatus. The genus *Aporcelaimoides* is restored, its diagnosis emended, and three species of *Sectonema*, namely *S. amazonicum*, *S. haguei* and *S. moderatum*, transferred to it. An updated list of its species, a key to their identification and a tabular compendium with the most important morphometric features are also presented.

Keywords

Description, morphology, morphometrics, new combinations, new species, taxonomy, *Sectonema*, SEM

Introduction

The genus *Aporcelaimoides* is an interesting aporcelaimoid taxon, created by Heyns (1965) to accommodate two new species, namely *A. probulbum* (type species) and *A. californicum*. It was originally characterized among other features by its "Spear dorylaimoid, with a large dorsal aperture, but the basal part of the spear much narrower than the lumen of the pharynx, and situated ventrally in the pharynx". Later, Siddiqi (1995) regarded it as a junior synonym of *Sectonema* Thorne, 1930, an action that, several years later, was followed by Andrássy (2009). However, this taxonomical decision deserves further analyses since important morphological differences exist between both genera, being especially important those affecting the nature of the stomatal protrusible structure.

The study of dorylaimid fauna from Vietnam has received poor attention. Several authors (Vu et al. 2010; Nguyen et al. 2011; Gagarin and Gusakov 2012 and 2013a,b; Nguyen et al. 2014) discovered some new species and reported for the first time information about other known species. It is suggested that Vietnamese dorylaimid fauna might be highly diverse. This is the first contribution in a series devoted to study the aporcelaimid fauna of this Asian country.

During a general nematological survey conducted during the last five years to study the diversity of the Vietnamese nematode fauna, several specimens of the genus *Aporcelaimoides* were collected from natural areas in Vietnam. Their detailed examination revealed they belonged to three unknown forms, which are herein described. Besides, the study of this nematode material has confirmed relevant data to reconsider the identity of the genus *Aporcelaimoides*.

Material and methods

Nematodes

Nematodes were collected from several natural areas in Vietnam, extracted from soil samples using the methods of Baermann (1917) and Flegg (1967) somewhat modified, relaxed and killed by heat, fixed in 4% formaldehyde, and processed to anhydrous glycerine following Siddiqi's (1964) technique. Finally, the specimens were mounted on permanent glass slides to allow handling and observation under LM.

Light microscopy

Nematodes were measured using a light microscope. Morphometrics included de Man's indices and most of the usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof and Coomans (1970) and spicule terminology follows Peña-Santiago et al. (2014). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe® Photoshop® CS. Drawings were made using a *camera lucida*.

Scanning electron microscopy

After their examination and identification, a few specimens preserved in glycerin were recycled to their observation under SEM following the protocol by Abolafia and Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope.

Taxonomy

***Aporcelaimoides brevistylum* sp. n.**
http://zoobank.org/0D05FA79-C8FB-46A8-9496-02F6F9D8E6E7
Figs 1–3, 4A–E

Material examined. Twelve females and fourteen males from two localities, in variable state of preservation.

Measurements. See Table 1.

Description. *Adult.* Moderately slender to slender nematodes of medium size, 1.95–2.90 mm long. Body cylindrical, distinctly tapering towards the anterior end, less so towards the posterior one because the caudal region is rounded. Habitus regularly (often strongly) curved ventrad after fixation, usually spiral-shaped. Cuticle three-layered, especially distinguishable at caudal region, where it consists of thinner outer layer bearing very fine transverse striation through the entire body, thicker intermediate layer with radial striation and thin inner layer; thickness 3–5 μm at anterior region, 4–7 μm in mid-body and 9.0–12.5 μm on tail. Lateral chord 8–20 μm wide at mid-body, occupying one-eighth to less than one-fifth (12–18%) of mid-body diameter. Three ventral and three dorsal body pores are usually present at level of mural odontostyle-odontophore, their corresponding ducts appearing especially thickened beneath intermediate cuticle layer. Lip region offset by deep constriction, 2.7–3.3 times as wide as high and one-fifth to two-sevenths (18–30%) of body diameter at neck base; lips (under SEM) amalgamated; labial papillae button-like, very perceptible and protruding under LM, surrounded by a ring-like annulus (occasionally two annuli), the inner ones at the margin of oral field; cephalic papillae larger than the labial ones, with an oval transverse slit; oral aperture a dorso-ventral, slightly hexagonal orifice, the lip region hence showing a biradial symmetry. Amphid fovea cup-shaped, its opening occupying 9–11 µm or one-half to two-thirds (52–64%) of lip region diameter. Cheilostom nearly cylindrical, lacking any differentiation. Mural odontostyle attached subventrally and comparatively short, 4.1–5.4 times as long as wide, 0.6–0.8 times as long as lip region diameter, and 0.43–0.61% of body length; aperture 8–9 µm long or up to five-sevenths (62–71%) its length. Guiding ring simple, somewhat plicate, at 0.6–0.8 lip region diameters from anterior end. Odontophore linear, rod-like, 3.4–4.2 times the mural odontostyle length. Anterior region of pharynx enlarging very gradually; basal expansion 9.5–12.6 times as long as wide, 4.6–7.2 times as long as body diameter, and occupying 58–66% of total neck length; gland nuclei obscure in most specimens examined, DN = 50 (n=1) and S_2N = 84 (n=1). Nerve ring located at 154–185 µm from anterior end or 21–26% of total neck length. Cardia rounded conoid, 10–14 × 14–18 µm; a ring-like structure is present surrounding its junction to pharyngeal base. Tail short and rounded; inner core with irregular shape at tail end. Caudal pores two pairs, one lateral, another sub-lateral.

Female. Genital system didelphic-amphidelphic, with both branches almost equally and well developed, the anterior 207–254 µm long or 9–10% of body length and the posterior 233–300 µm long or 9–13% of body length. Ovaries moderately sized, usually not surpassing the sphincter level, the anterior 95–365 µm, the posterior 106–316 µm long; oocytes arranged first in two or more rows, then in a single row. Oviduct 96–124 µm long or 1.0–1.4 times the corresponding body diameter, and consisting of a slender part with prismatic cells and a well developed *pars dilatata* bearing wide lumen that often containing sperm cells inside. Oviduct-uterus junction marked by a sphincter. Uterus a short, simple, tube-like structure 85–182 µm long or 1.0–2.1 times the corresponding body diameter, most specimens with abundant sperm cells inside. Uterine eggs ovoid, 153 (n=1) × 79, 85 (n=2) µm, 1.8 (n=1) times as long as wide. Vagina extending inwards 43–57 µm or four-ninths to two-thirds (45–65%) of body diameter: *pars proximalis* 32–44 × 28–34 µm, with somewhat sigmoid walls and surrounded by weak musculature; *pars refringens* absent; and *pars distalis* well developed, 11–14 µm long. Vulva a post-equatorial transverse slit. Prerectum 1.8–2.6, rectum 1.0–1.2 anal body diameters long.

Male. Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 15–20 µm from cloacal aperture, there is only one ventromedian supplement located out the range of spicules, at 48, 58 (n=2) µm from ad-cloacal pair. Spicules distinctly robust and massive, especially in its posterior half, 3.4–4.6 times its maximum width, 1.2–1.7 times the body diameter at level of the cloacal aperture:

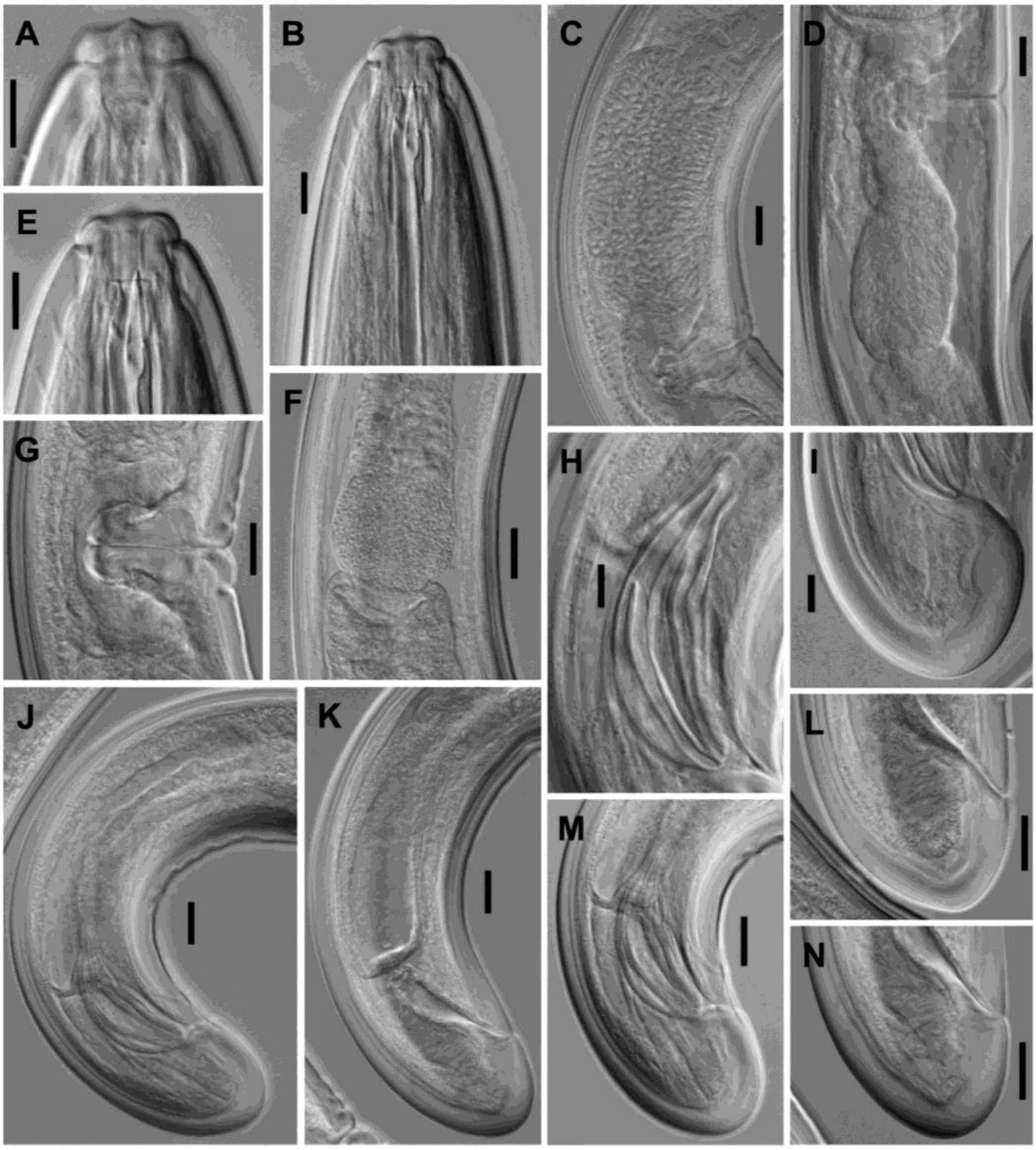

Figure 2. *Aporcelaimoides brevistylum* sp. n. (LM, type population). **A** Anterior region in surface, lateral view **B, E** Anterior region in median, lateral view **C, D** Uterus, containing sperm cells inside **F** Pharyngo-intestinal junction **G** Vagina **H** Spicule **I, M** Male, caudal region **J** Male, posterior body region **K** Female, posterior body region **L, N** Female, caudal region. Scale bars: 10 µm (**A, B, E, H, I**); 20 µm (**C, D, F, G, J–N**).

dorsal contour regularly convex, ventral contour very weakly concave, with shallow or weak hump and hollow; curvature 126–142°; head occupying 7–21% of spicule total length, its dorsal contour conspicuously curved at its anterior end and longer than the ventral one, which is short and straight; median piece 7.2–10.9 times as long as wide, occupying 35–50% of spicule maximum width, reaching the posterior tip; posterior end 5–9 µm wide. Lateral guiding pieces 13–17 µm long, 3.5–5.1 times as long as wide. Prerectum 2.9–4.4, cloaca 1.1–1.3 the corresponding body widths long.

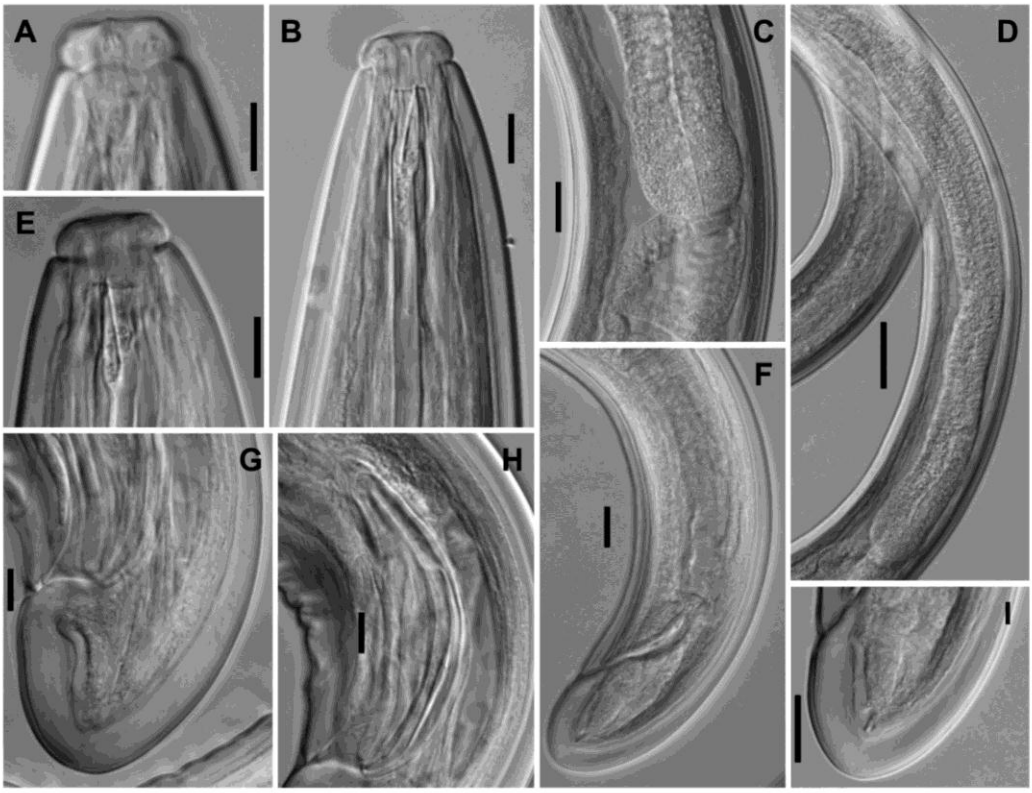

Figure 3. *Aporcelaimoides brevistylum* sp. n. (LM, other population). **A** Anterior region in surface, lateral view **B, E** Anterior region in median, lateral view **C** Pharyngo-intestinal junction **D** Pharyngeal expansion **F** Female, posterior body region **G** Male, caudal region **H** Spicules **I** Female, caudal region. (Scale bars: 10 µm (**A, B, E, G, H**); 20 µm (**C, F, I**); 50 µm (**D**).

Diagnosis. The new species is characterized by its body 1.95–2.90 mm long, lip region offset by deep constriction and 17–18 µm broad, ventral side of mural odontostyle 11–14 µm with aperture occupying 62–71% of its length, neck 663–767 µm long, pharyngeal expansion 387–508 µm long or occupying 58–66% of total neck length, uterus a simple tube and 85–182 µm long or 1.0–2.1 times the corresponding body diameter, *pars refringens vaginae* absent, $V = 55–63$, female tail short and rounded (35–46 µm, $c = 58–76$, $c' = 0.6–0.8$), male tail similar to that of female (34–42 µm, $c = 49–69$, $c' = 0.6–0.8$), spicules 67–86 µm long, and one ventromedian supplement bearing hiatus.

Relationships. In having short mural odontostyle (11–14 µm at its ventral side) and *pars refringens vaginae* absent, the new species is morphologically close to *A. californicum* Heyns, 1965 and *A. probulbum* Heyns, 1965, but it can be distinguished from both species in its smaller ($L = 1.95–2.90$ *vs* $L =$ more than 3) and less slender ($a = 25–35$ *vs* $a \geq 41$) body. Besides, *A. brevistylum* sp. n. differs from *A. californicum* in its comparatively longer neck ($b = 3.3–3.7$ *vs* $b = 7.6$), larger mural odontostyle aperture (occupying 62–71% *vs* one-half of its length), more posterior vulva ($V = 55–63$ *vs* $V = 51$), shorter uterus (85–182 µm or 1.0–2.1 times the corresponding body diameter *vs*

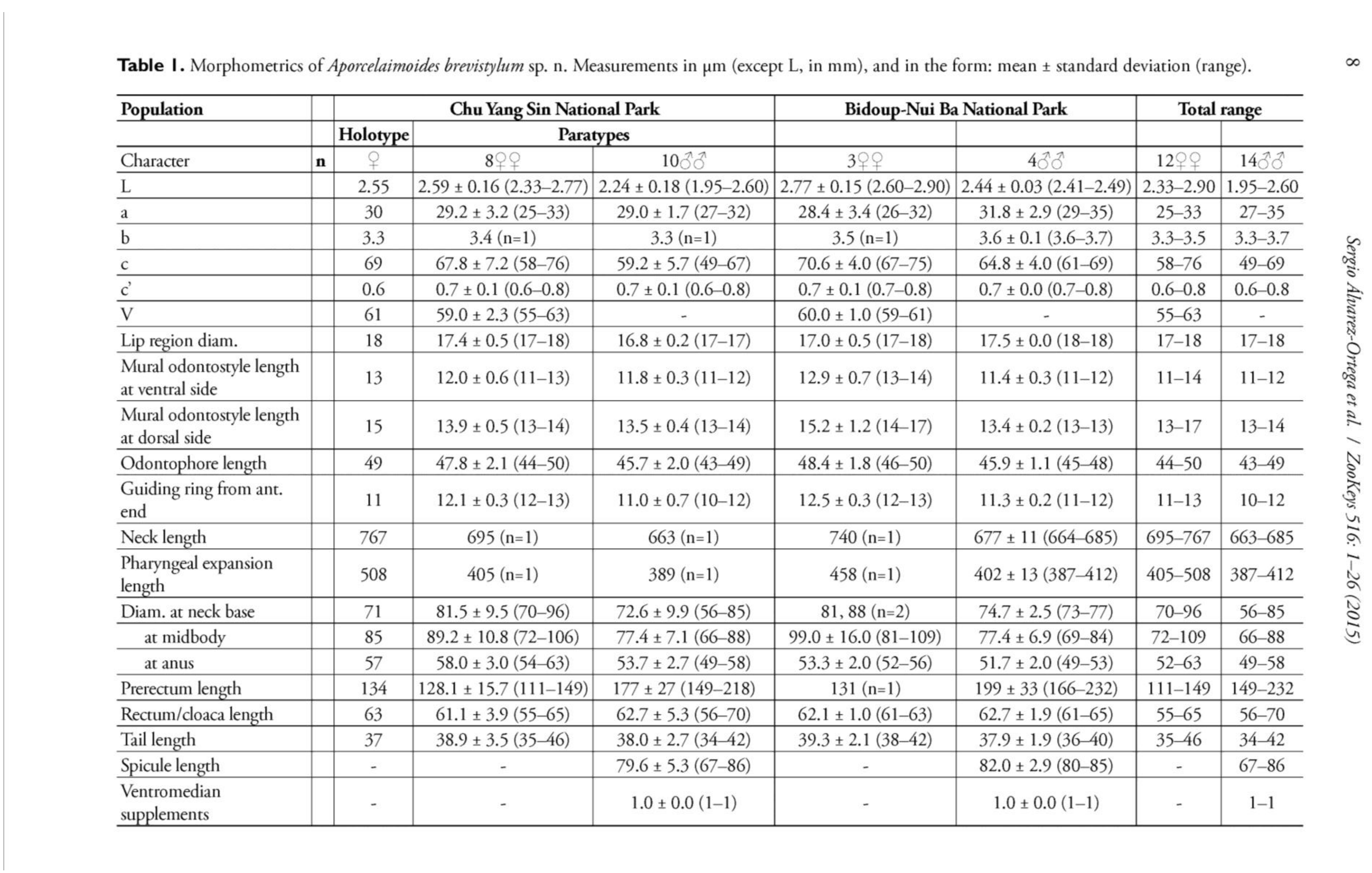

Table 1. Morphometrics of *Aporcelaimoides brevistylum* sp. n. Measurements in µm (except L, in mm), and in the form: mean ± standard deviation (range).

Population		Chu Yang Sin National Park			Bidoup-Nui Ba National Park		Total range	
		Holotype	Paratypes					
Character	n	♀	8♀♀	10♂♂	3♀♀	4♂♂	12♀♀	14♂♂
L		2.55	2.59 ± 0.16 (2.33–2.77)	2.24 ± 0.18 (1.95–2.60)	2.77 ± 0.15 (2.60–2.90)	2.44 ± 0.03 (2.41–2.49)	2.33–2.90	1.95–2.60
a		30	29.2 ± 3.2 (25–33)	29.0 ± 1.7 (27–32)	28.4 ± 3.4 (26–32)	31.8 ± 2.9 (29–35)	25–33	27–35
b		3.3	3.4 (n=1)	3.3 (n=1)	3.5 (n=1)	3.6 ± 0.1 (3.6–3.7)	3.3–3.5	3.3–3.7
c		69	67.8 ± 7.2 (58–76)	59.2 ± 5.7 (49–67)	70.6 ± 4.0 (67–75)	64.8 ± 4.0 (61–69)	58–76	49–69
c'		0.6	0.7 ± 0.1 (0.6–0.8)	0.7 ± 0.1 (0.6–0.8)	0.7 ± 0.1 (0.7–0.8)	0.7 ± 0.0 (0.7–0.8)	0.6–0.8	0.6–0.8
V		61	59.0 ± 2.3 (55–63)	-	60.0 ± 1.0 (59–61)	-	55–63	-
Lip region diam.		18	17.4 ± 0.5 (17–18)	16.8 ± 0.2 (17–17)	17.0 ± 0.5 (17–18)	17.5 ± 0.0 (18–18)	17–18	17–18
Mural odontostyle length at ventral side		13	12.0 ± 0.6 (11–13)	11.8 ± 0.3 (11–12)	12.9 ± 0.7 (13–14)	11.4 ± 0.3 (11–12)	11–14	11–12
Mural odontostyle length at dorsal side		15	13.9 ± 0.5 (13–14)	13.5 ± 0.4 (13–14)	15.2 ± 1.2 (14–17)	13.4 ± 0.2 (13–13)	13–17	13–14
Odontophore length		49	47.8 ± 2.1 (44–50)	45.7 ± 2.0 (43–49)	48.4 ± 1.8 (46–50)	45.9 ± 1.1 (45–48)	44–50	43–49
Guiding ring from ant. end		11	12.1 ± 0.3 (12–13)	11.0 ± 0.7 (10–12)	12.5 ± 0.3 (12–13)	11.3 ± 0.2 (11–12)	11–13	10–12
Neck length		767	695 (n=1)	663 (n=1)	740 (n=1)	677 ± 11 (664–685)	695–767	663–685
Pharyngeal expansion length		508	405 (n=1)	389 (n=1)	458 (n=1)	402 ± 13 (387–412)	405–508	387–412
Diam. at neck base		71	81.5 ± 9.5 (70–96)	72.6 ± 9.9 (56–85)	81, 88 (n=2)	74.7 ± 2.5 (73–77)	70–96	56–85
at midbody		85	89.2 ± 10.8 (72–106)	77.4 ± 7.1 (66–88)	99.0 ± 16.0 (81–109)	77.4 ± 6.9 (69–84)	72–109	66–88
at anus		57	58.0 ± 3.0 (54–63)	53.7 ± 2.7 (49–58)	53.3 ± 2.0 (52–56)	51.7 ± 2.0 (49–53)	52–63	49–58
Prerectum length		134	128.1 ± 15.7 (111–149)	177 ± 27 (149–218)	131 (n=1)	199 ± 33 (166–232)	111–149	149–232
Rectum/cloaca length		63	61.1 ± 3.9 (55–65)	62.7 ± 5.3 (56–70)	62.1 ± 1.0 (61–63)	62.7 ± 1.9 (61–65)	55–65	56–70
Tail length		37	38.9 ± 3.5 (35–46)	38.0 ± 2.7 (34–42)	39.3 ± 2.1 (38–42)	37.9 ± 1.9 (36–40)	35–46	34–42
Spicule length		-	-	79.6 ± 5.3 (67–86)	-	82.0 ± 2.9 (80–85)	-	67–86
Ventromedian supplements		-	-	1.0 ± 0.0 (1–1)	-	1.0 ± 0.0 (1–1)	-	1–1

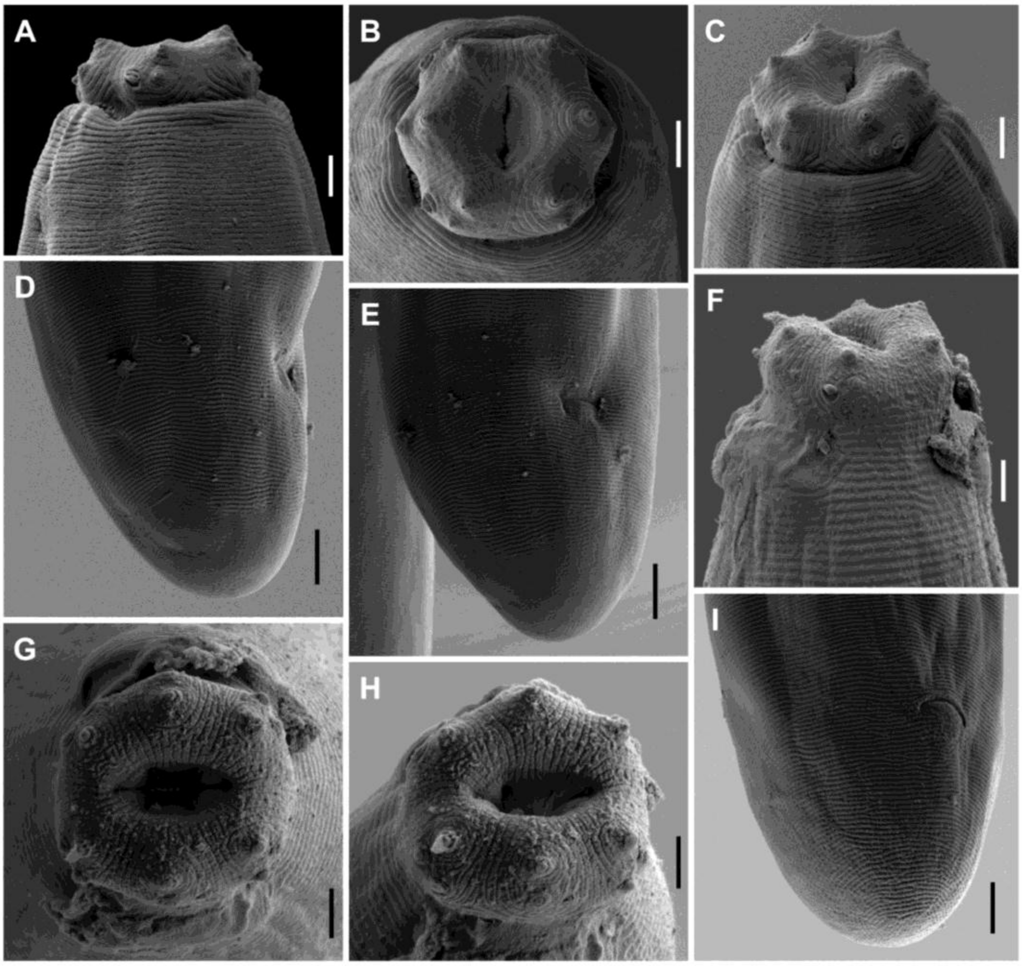

Figure 4. *Aporcelaimoides brevistylum* sp. n. **A–E** and *A. silvaticum* sp. n. **F–I** (SEM, juvenile). **A, C, F** Lip region in ventral view **B, G, H** Lip region in face view **D, E, I** Caudal region in lateral (**D**) or subventral (**E, F**) view. Scale bars: 2 μm (**A–C, F–H**); 5 μm (**D, E, I**).

about 430 μm long or about 5.3 times the corresponding body diameter), comparatively shorter female tail (c = 58–76, c' = 0.6–0.8 *vs* c = 126, c' = 1.0), and male present (*vs* absent). And from *A. probulbum* in its shorter neck (663–767 μm, b = 3.3–3.7 *vs* 883–1011 μm, b = 3.9–5.2), narrower lip region (17–18 *vs* about 21 μm), and comparatively longer tail (c = 49–76 *vs* c = 75–127).

Moreover, in having short mural odontostyle (11–14 μm at its ventral side) the new species resembles *A. haguei* (Hunt, 1978), comb. n., but it differs in its smaller general size (L = 1.95–2.90 and neck 663–767 μm long *vs* L = 4.67–5.42 and neck 1172–1178 μm long), less slender body (a = 25–35 *vs* a = 52–62), absence (*vs* presence of rows of minute denticles on stomatal wall, indeed a very relevant feature), *pars refringens vaginae* absent (*vs* present), comparatively longer female tail (c = 49–76 *vs* c = 99–118), and male present (*vs* absent).

Type locality and habitat. Vietnam, Dak Lak province, Chu Yang Sin National Park, where it was collected from soil of a pristine forest in October 2012.

Other locality and habitat. Vietnam, Lam Dong Province, Bidoup-Nui Ba National Park, from soil of a pristine forest, collected in June 2013.

Type material. Female holotype and seven female and nine male paratypes deposited in the nematode collection of the University of Jaén, Spain. One female and one male paratypes deposited in the nematode collection of the Institute of Ecology and Biological Resources, Vietnam.

Etymology. The specific epithet is a compound Latin term referring to the short mural odontostyle that characterizes this species.

Remarks. The two populations examined are very similar in their morphological features and morphometrics, but some minor differences have been also noted, which are herein regarded as intraspecific variation. Thus, the population from Dak Lak province shows a shorter mural odontostyle (ventral side 11–13 *vs* 13–14 µm, dorsal side 13–14 *vs* 14–17 µm, in females) and comparatively longer neck (*b* = 3.3–3.4 *vs* *b* = 3.5–3.7).

Aporcelaimoides minor sp. n.

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Figs 5–7

Material examined. Ten females from three localities, in good state of preservation.

Measurements. See Table 2.

Description. *Female.* Moderately slender to slender nematodes of medium size, 2.09–2.61 mm long. Body cylindrical, distinctly tapering towards the anterior end, less so towards the posterior one as the caudal region is very short and rounded to truncate. Habitus regularly curved ventrad after fixation, often spiral-shaped. Cuticle three-layered, especially distinguishable at caudal region: thin outer layer bearing fine transverse striation through the entire body, a much thicker intermediate layer with radial striation, and a thin inner layer; thickness 3.0–4.5 µm at anterior region, 4.5–6.5 µm in mid-body and 6.5–9.5 µm on tail. Lateral chord 7–13 µm wide at mid-body, occupying one-tenth to less than one-sixth (9–15%) of mid-body diameter. Two ventral and two dorsal body pores are usually present at level of mural odontostyle-odontophore, their corresponding ducts appearing especially thickened beneath intermediate cuticle layer. Lip region offset by deep constriction, 2.8–3.3 times as wide as high and one-fifth to less than one-third (21–30%) of body diameter at neck base; lips mostly amalgamated, somewhat angular; papillae perceptible, somewhat protruding. Amphid fovea cup-shaped, its opening occupying 8–10 µm or up to one-half (44–50%) of lip region diameter. Cheilostom nearly cylindrical, lacking any differentiation. Mural odontostyle attached subventrally, 6.7–7.7 times as long as wide, 0.7–0.9 times as long as lip region diameter, and 0.54–0.72% of body length; aperture 11–13 µm long or up to six-sevenths (73–84%) its length. Guiding ring simple, somewhat plicate, at 0.6–0.8

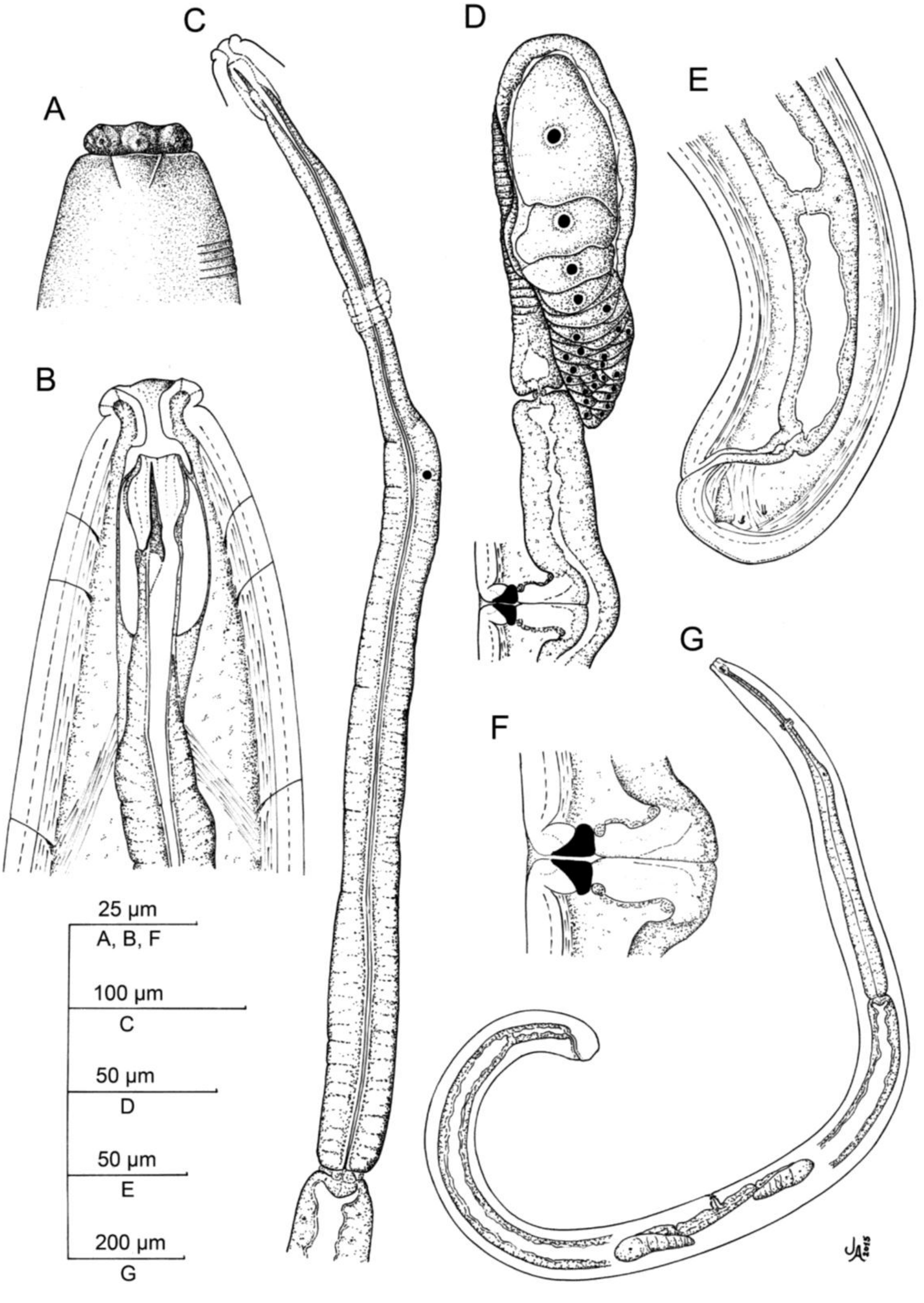

Figure 5. *Aporcelaimoides minor* sp. n. (Female, line drawing). **A** Lip region in surface, lateral view **B** Anterior region in median, lateral view **C** Neck **D** Anterior genital branch and vagina **E** Posterior body region **F** Vagina **G** Entire.

Table 2. Morphometrics of *Aporcelaimoides minor* sp. n. and *A. silvaticum* sp. n. Measurements in µm (except L, in mm), and in the form: mean ± standard deviation (range).

Species		***A. minor* sp. n.**					***A. silvaticum* sp. n.**		
Population		**Tay Yen Tu Natural Reserve**		**Cao Bang Natural Reserve**	**Chu Yang Sin National Park**	**Total Range**	**Cuc Phuong National Park**		
		Holotype	**Paratypes**				**Holotype**	**Paratypes**	
Character	n	♀	2♀♀	2♀♀	5♀♀	10♀♀	♀	♀	4♂♂
L		2.12	2.09, 2.24	2.31, 2.17	2.46 ± 0.14 (2.29–2.61)	2.09–2.61	2.60	2.56	2.31 ± 0.24 (2.09–2.58)
a		24	?, 23	29, 26	28.8 ± 2.9 (26–33)	23–33	29	?	30.7 ± 2.1 (28–33)
b		3.3	3.3, 3.6	4.0, 3.5	3.9 ± 0.3 (3.7–4.4)	3.3–4.4	3.6	3.8	3.7 ± 0.4 (3.4–4.2)
c		117	145, 98	142, 146	109.0 ± 17.2 (90–137)	90–146	83	69	79.9 ± 10.8 (67–94)
c’		0.3	0.3, 0.4	0.3 (n=2)	0.5 ± 0.1 (0.3–0.6)	0.3–0.6	0.7	0.7	0.6 ± 0.0 (0.6–0.7)
V		55	56, 52	50, 53	109.0 ± 17.2 (90–137)	48–56	60	58	-
Lip region diam.		19	20, 19	20, 19	19.4 ± 0.7 (19–20)	19–20	17	17	17.2 ± 0.3 (17–18)
Mural odontostyle length at ventral side		14	15 (n=2)	17, 16	14.3 ± 0.6 (14–15)	14–16	12	12	11.3 ± 0.2 (11–12)
Mural odontostyle length at dorsal side		15	16 (n=2)	14, 15	16.0 ± 0.7 (15–17)	15–17	13	13	12.7 ± 0.2 (12–13)
Odontophore length		33	33 (n=2)	33 (n=2)	33.2 ± 0.7 (33–34)	33–34	47	46	40.3 ± 1.3 (39–41)
Guiding ring from ant. end		14	15 (n=2)	14, ?	12.7 ± 1.0 (12–14)	12–15	12	12	10.9 ± 2.0 (9–13)
Neck length		646	632, 630	579, 618	626 ± 28 (592–649)	579–649	720	668	625 ± 40 (597–684)
Pharyngeal expansion length		423	400, 380	331, 382	391 ± 27 (360–420)	331–423	452	400	383 ± 37 (353–436)
Diam. at neck base		81	80, 90	67, 74	83.9 ± 9.7 (69–93)	67–93	87	?	71.9 ± 5.8 (65–79)
at midbody		87	?, 97	80, 83	86.0 ± 9.6 (70–95)	70–97	88	?	75.1 ± 4.8 (69–80)
at anus		57	51 (n=2)	49, 55	51.2 ± 5.4 (46–60)	46–60	48	51	46.3 ± 1.1 (45–48)
Prerectum length		79	76, ?	73, 95	97.0 ± 9.5 (87–106)	73–106	117	114	148.4 ± 5.6 (142–153)
Rectum/cloaca length		47	50 (n=2)	50, 42	52.7 ± 5.3 (47–60)	42–60	55	42	61.5 ± 4.9 (56–66)
Tail length		18	14, 23	16, 15	22.9 ± 3.4 (17–26)	14–26	31	37	29.1 ± 2.4 (27–31)
Spicule length		-	-	-	-	-	-	-	70.4 ± 5.1 (64–75)
Ventromedian supplements		-	-	-	-	-	-	-	2.5 ± 0.6 (2–3)

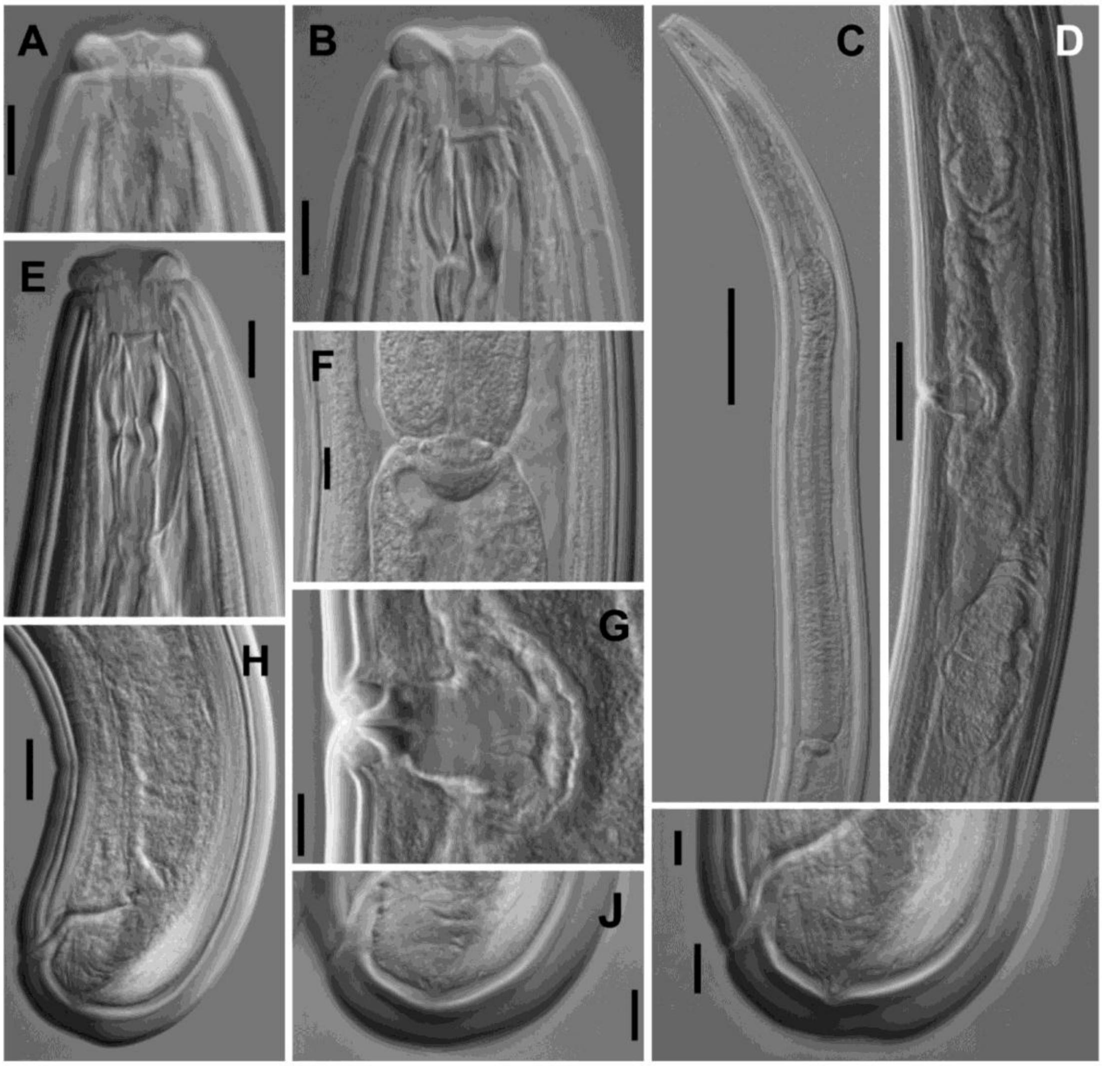

Figure 6. *Aporcelaimoides minor* sp. n. (LM, female, type population). **A** Anterior region in surface, lateral view **B, E** Anterior region in median, lateral view **C** Neck region **D** Genital system **F** Pharyngo-intestinal junction **G** Vagina **H** Posterior body region **I, J** Caudal region. Scale bars: 10 µm (**A, B, E–G, I, J**); 100 µm (**C**); 50 µm (**D**); 20 µm (**H**).

vs 17–18 µm), smaller mural odontostyle aperture (occupying 73–84% *vs* 62–71% its length), shorter female tail (14–26 µm, c = 90–146, c' = 0.3–0.6 *vs* 35–46 µm, c = 58–76, c' = 0.6–0.8), and male absent (*vs* present). And from *A. californicum* in its shorter (L = 2.09–2.61 *vs* L = 5.53) and less slender (a = 23–33 *vs* a = 75) body, larger mural odontostyle aperture (occupying 73–84% *vs* one-half of its length), shorter uterus (44–69 µm long or less than one body diameter *vs* about 430 µm long or about 5.3 times the corresponding body diameter), and shorter female tail (14–26 µm, c' = 0.3–0.6 *vs* 44 µm, c' = 1.0).

Type locality and habitat. Vietnam, Bac Giang Province, Tay Yen Tu Natural Reserve, collected from soil in a pristine tropical forest, in July 2008.

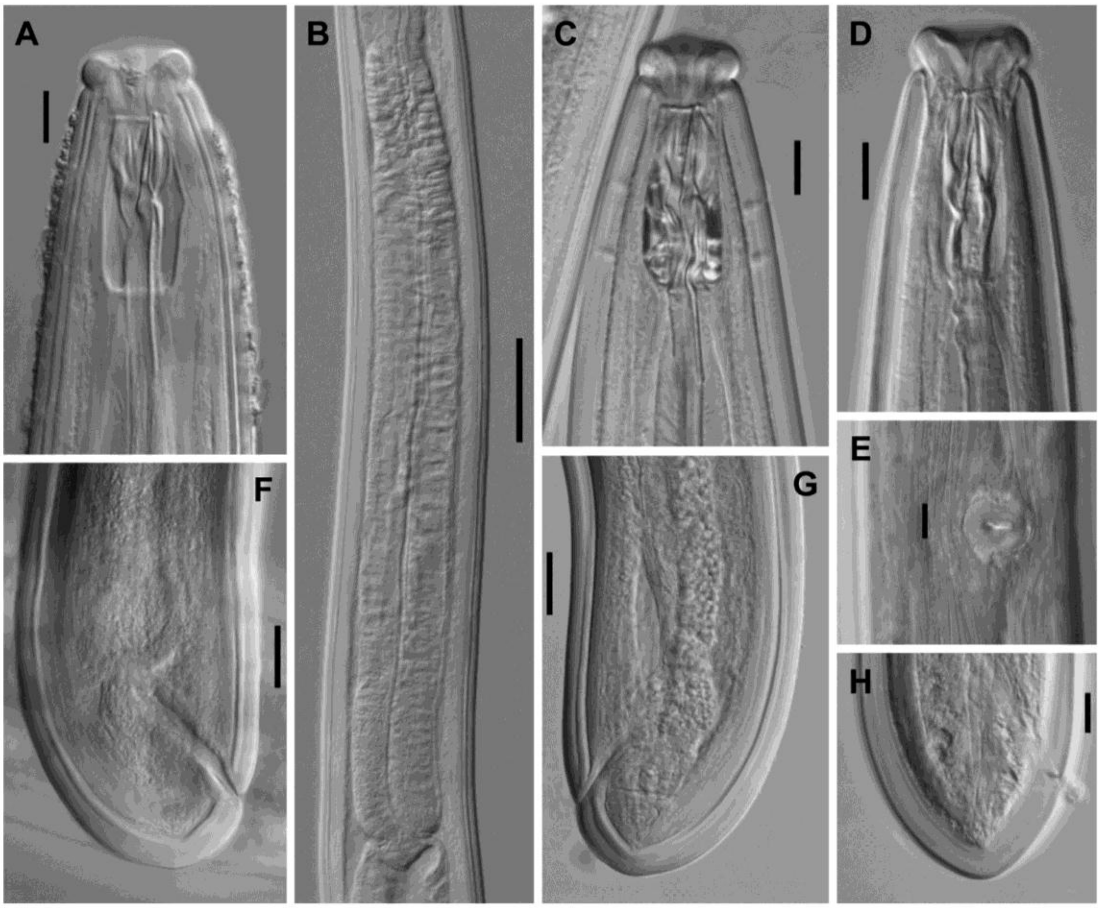

Figure 7. *Aporcelaimoides minor* sp. n. (LM, female, other populations). **A, B, F** (Population from Cao Bang Natural Reserve) **C–E, G, H** (Population from Chu Yang Sin National Park) **A, C** Anterior region in median, lateral view **B** Pharyngeal expansion **D** Anterior region in median, ventral view **E** Vulva in ventral view **F–H** Caudal region. Scale bars: 10 µm (**A, C, D, E, H**); 50 µm (**B**); 20 µm (**F, G**).

Other localities and habitats. Vietnam, Cao Bang Province, Cao Bang Natural Reserve (GPS coordinates: 22°34'07"N and 105°52'34"), in a tropical evergreen forest soil in association with *Dipterocarpus* sp. and *Cinnamomum* sp., collected in 2013. Dak Lak province, Chu Yang Sin National Park, in October 2012.

Type material. Female holotype and one female paratype deposited in the nematode collection of the University of Jaen, Spain. One female paratype deposited in the nematode collection of the Institute of Ecology and Biological Resources, Vietnam.

Etymology. The specific epithet means 'small' and refers to the comparatively small general size of the new species.

Remarks. In spite of it was collected from three localities, the material examined is very similar in its main morphological features and morphometrics. Nevertheless, some differences have been also observed, especially affecting the female tail shape as some specimens show a short and rounded-conoid caudal region whereas it becomes extremely short and truncate in other individuals.

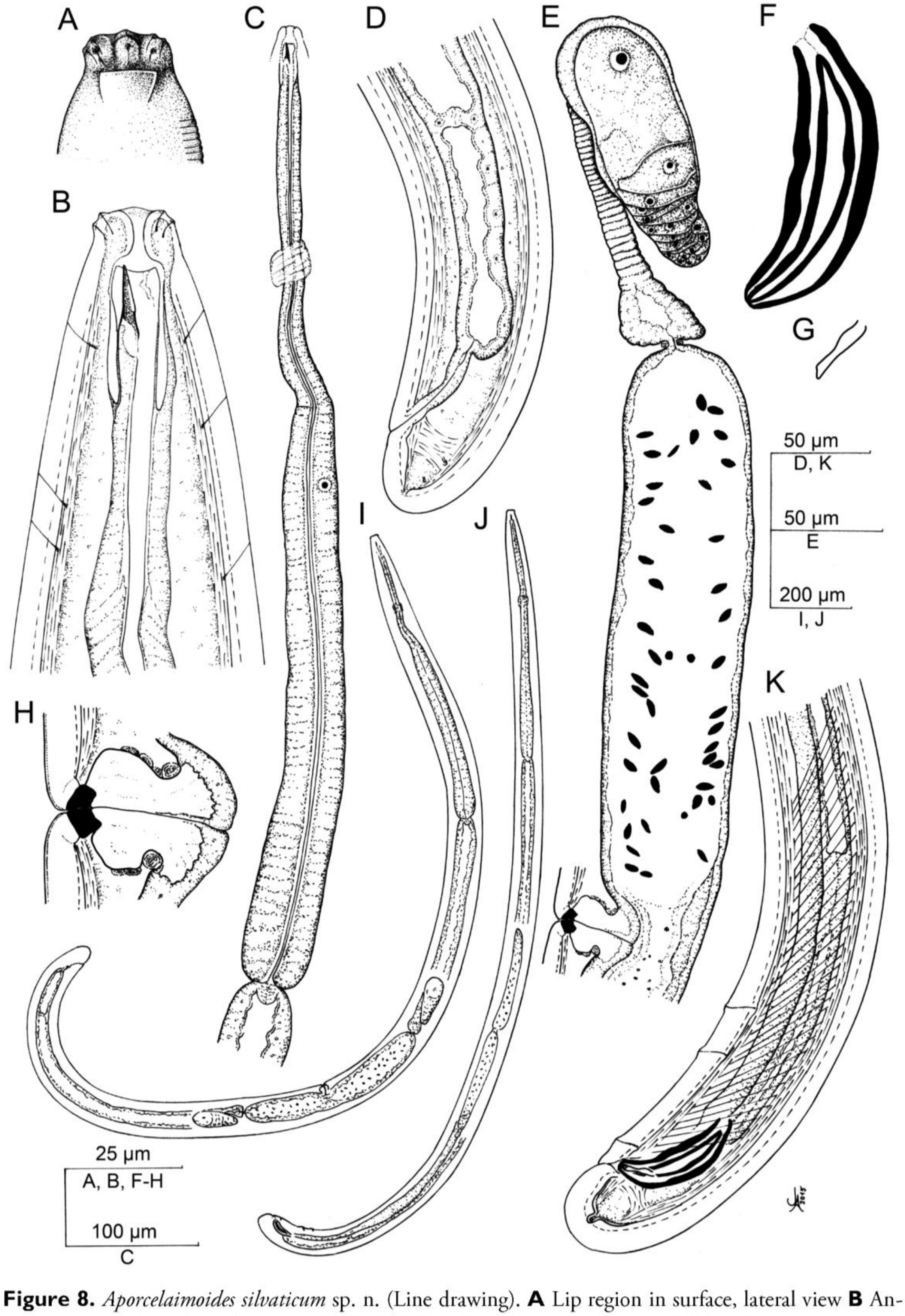

Figure 8. *Aporcelaimoides silvaticum* sp. n. (Line drawing). **A** Lip region in surface, lateral view **B** Anterior region in lateral, median view **C** Neck **D** Female, posterior body region **E** Female, anterior genital branch and vagina **F** Spicule **G** Lateral guiding piece **H** Vagina **I** Female, entire **J** Male, entire **K** Male, posterior body region.

103 µm long; oocytes arranged first in two or more rows, then in a single row. Oviduct 99–107 µm long or 1.2, 1.3 times the corresponding body diameter, and consisting of a slender part with prismatic cells and a moderately developed *pars dilatata* with visible lumen but no sperm cell. Oviduct-uterus junction marked by a sphincter. Uterus a short, simple, tube-like structure 128–243 µm long or 2.1, 2.8 times the corresponding body diameter, one female containing abundant sperm cells inside. Vagina extending inwards 31, 32 µm or about three-eighths (36%, n=1) of body diameter: *pars proximalis* 23, 24 × 25, 26 µm, with somewhat sigmoid walls and surrounded by weak musculature; *pars refringens* with two small, triangular to drop-shaped pieces measuring 5 × 4, 5 µm and with a combined width of 9, 10 µm; and *pars distalis* 1.0, 1.5 µm long. Vulva a post-equatorial transverse slit. Prerectum 2.3, 2.5, rectum 0.8, 1.2 anal body diameters long.

Male. Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 16–19 µm from cloacal aperture, there is a series of two or three widely spaced (22–42 µm apart) ventromedian supplements, the posteriormost of which lying out the range of spicules, but very close to the spicules end, being situated at 42–63 µm from ad-cloacal pair. Spicules relatively robust, 3.5–4.6 times its maximum width, 1.3–1.6 times the body diameter at level of the cloacal aperture: dorsal contour regularly convex, ventral contour bearing weak hump and hollow; curvature 140–143°; head occupying 8–10% of spicule total length, with both contours nearly straight, and its dorsal side longer than the ventral one; median piece 6.5–8.3 times as long as wide, occupying 45–54% of spicule maximum width, reaching the posterior tip; posterior end 5–6 µm wide. Lateral guiding pieces 19–23 µm long, 6.4–7.8 times as long as wide. Prerectum 3.0–3.3, cloaca 1.2–1.4 times the corresponding body width long.

Diagnosis. The new species is characterized by its body 2.09–2.60 mm long, lip region offset by depression and 17–18 µm broad, mural odontostyle 11–12 µm long at its ventral side with aperture occupying 60–66% of its length, neck 597–720 µm long, pharyngeal expansion 353–452 µm long or occupying 58–64% of total neck length, uterus a simple tube and 128–243 µm long or 2.1–2.8 times the corresponding body diameter, *pars refringens vaginae* well developed, $V = 58–60$, female tail short and rounded to rounded conoid (31–37 µm, $c = 69–83$, $c' = 0.7$), male tail similar to that of female (27–31 µm, $c = 67–94$, $c' = 0.6–0.7$), spicules 64–75 µm long, and two or three widely spaced ventromedian supplements bearing hiatus.

Relationships. The new species differs from its relatives by its lip region offset by depression (*vs* constriction). Besides, in having *pars refringens vaginae* and short mural odontostyle (11–12 µm long at its ventral side), *A. silvaticum* sp. n. is morphologically similar to *A. haguei* comb. n. and *A. minor* sp. n., but it can be distinguished from them in its narrower lip region (17–18 *vs* equal or 19 µm or more) and more posterior vulva ($V = 58–60$ *vs* V up to 57). It also differs from *A. haguei* comb. n. in its smaller general size ($L = 2.09–2.60$, neck 597–720 µm long *vs* $L = 4.67–5.42$, neck 1112–1178 µm long), less slender body ($a = 28–33$ *vs* $a = 52–62$), absence (*vs* presence) of rows of minute denticles on stomatal wall), shorter female tail (31–37 *vs* 46–47 µm), and male present (*vs* absent). And from *A. minor* sp. n. in its shorter mural odontostyle

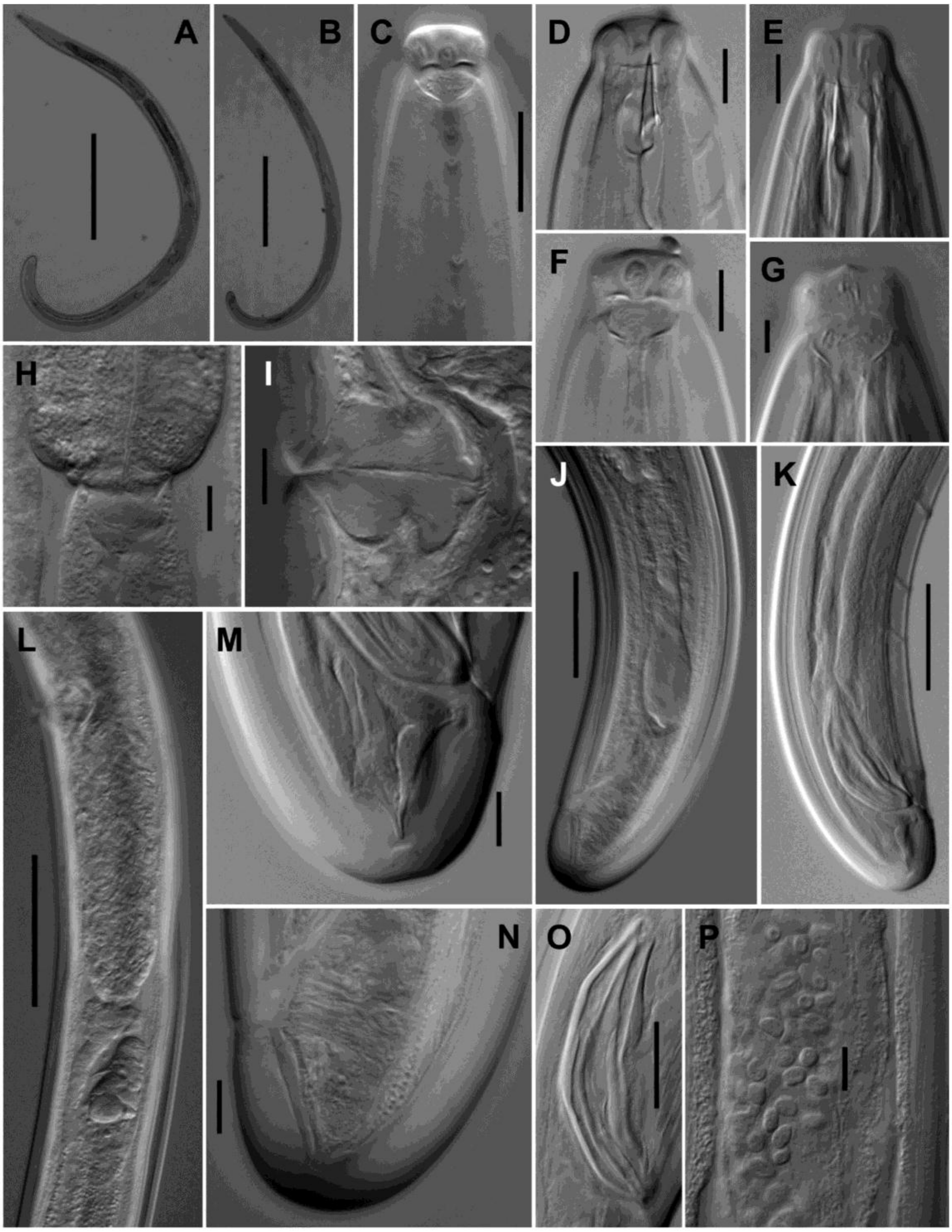

Figure 9. *Aporcelaimoides silvaticum* sp. n. (LM). **A** Female, entire **B** Male, entire **C** Anterior region in surface, lateral view showing four lateral pores **D, E** Anterior region in median, lateral view **F, G** Anterior region in surface, lateral view showing the amphid fovea **H** Pharyngo-intestinal junction **I** Vagina **J** Female, posterior body region **K** Male, posterior body region **L** Female, posterior genital branch **M** Male, caudal region **N** Female, caudal region **O** Spicules **P** Sperm cells. Scale bars: 500 μm (**A, B**); 20 μm (**C**); 10 μm (**D–F, H, I, M–P**); 5 μm (**G**); = 50 μm (**J–L**).

(11–12 *vs* 14–16 μm at its ventral side) with smaller aperture (occupying 60–66% *vs* 73–84% of its length), longer female tail (27–31 μm, *c* = 67–94, *c'* = 0.6–0.7 *vs* 14–26 μm, *c* = 90–146, *c'* = 0.3–0.6), and male present (*vs* absent). Finally, the new species also resembles *A. brevistylum* sp. n. in having short mural odontostyle, but it differs from this in its well developed (*vs* absent) *pars refringens vaginae* and higher number of ventromedian supplements (two or three *vs* one).

Type locality and habitat. Northern Vietnam, Cuc Phuong National Park, where the new species was collected from soil of a pristine tropical forest in 2009.

Type material. Female holotype and one female and three male paratypes, deposited in the nematode collection of the University of Jaen, Spain. One male paratype deposited in the nematode collection of the Institute of Ecology and Biological Resources, Vietnam.

Etymology. The specific epithet is a Latin term meaning 'from the jungle', and refers to the habitat where the species dwells.

On the taxonomy of *Aporcelaimoides*

As mentioned in the introductory section, the identity of *Aporcelaimoides* has been matter of some controversy. In his original description of this genus, Heyns (1965) highlighted the differences between it and *Sectonema*, mainly based on the nature of the stomatal protrusible structure, "a dorylaimid spear which is set ventrally … similar in position to the mural tooth of *Sectonema*", putting hence emphasis on the existence of a large dorsal aperture in the protrusible structure of *Aporcelaimoides* and the absence of a such aperture in the mural tooth of *Sectonema*. Heyns (*op. cit.*) also noted that "the basal part of the spear (is) much narrower than the lumen of the pharynx (stoma)" [text between brackets incorporated by the authors]. Subsequent contributions by Andrássy (1976) and Jairajpuri and Ahmad (1992) assumed Heyns' point of view. Siddiqi (1995), however, stated (p. 99) that "…Since there is a great variation in the mural tooth of *Sectonema* (see Siddiqi 1984), there is no justification for holding *Aporcelaimoides* as a valid genus". And, later, Andrássy (2009) followed Siddiqi's opinion.

Very recently, Peña-Santiago and Álvarez-Ortega (2014a) redescribed *S. ventrale* Thorne, 1930, the type species of *Sectonema*, and conclude that (p. 1103) "the protrusible structure of *Sectonema*, as observed in its type species, is not a typical mural tooth as seen in nygolaims, but a reduced odontostyle with its base occupying most (if not whole) the stomatal lumen". It means that mural odontostyle of *Aporcelaimoides* significantly differs from the reduced odontostyle of *Sectonema*. Thus, Siddiqi's (1995) action might be not well supported as there are morphological arguments to separate both genera and to restore *Aporcelaimoides* as valid genus. Unfortunately, there is no molecular information of the latter, which would be especially useful to confirm the morphological data.

A revised diagnosis of *Aporcelaimoides* as well an updated list of its species, three of them transferred from *Sectonema*, and a key to their identification are given in the following. Besides, a compendium of their main morphometrics is presented in Table 3.

Table 3. Main morphometrics and distribution data of species belonging to the genus *Aporcelaimoides* Heyns, 1965 (Measurements in µm, except L, in mm).

	Character** Species	n	L	a	b	c	c'	V	Lrd	Mural Odont. Vent.	Mural Odont. Dors.	Neck	Ph. exp.	Tail	Spicul.	Ve. Sup.	Geog. Dis.	Reference
1	***amazonicum*** comb. n.	♀	3.34	43	3.4	88	0.8	53	27	25	30	991*	62%	38*	-	-	Brazil	Sidiqqi 1995
		♂	3.30	53	4.5	81	1.0	-	21*	?	23	733*	?	41*	78	3		
2	***brevistylum*** sp. n.	12♀♀	2.33–2.90	25–33	3.3–3.5	58–76	0.6–0.8	55–63	17–18	11–14	13–17	695–767	58–66%	35–46	-	-	Vietnam	Present paper
		14♂♂	1.95–2.60	27–35	3.3–3.7	49–69	0.6–0.8	-	17–18	11–12	13–14	663–685	58–60%	34–42	67–86	1		
3	***californicum***	♀	5.53	75	7.6	126	1.0	51	19*	16*	17	728*	?	44*	-	-	California-USA	Heyns 1965
4	***haguei*** comb. n.	2♀♀	4.67–5.42	52–62	4.2–4.6	99–118	0.7	56–57	24*	?	16	1112–1178*	71%*	46–47*	-	-	St. Lucia	Hunt 1978
5	***minor*** sp. n.	10♀♀	2.09–2.61	23–33	3.3–4.4	90–146	0.3–0.6	48–56	19–20	14–16	15–17	579–649	57–66%	14–26	-	-	Vietnam	Present paper
6	***moderatum*** comb. n.	2♀♀	4.34–5.66	37–38	3.9–4.2	82–105	0.7	55–59	25*	?	25–26.5	1113–1348*	?	53–54*	-	-	Cameroon	Siddiqi 1995
7	***probulbum***	11♀♀	3.35–4.75	41–55	3.9–5.2	75–127	0.6*	50–60	21*	15.5*	17–20	883*	64–67%	33*	-	-	South Africa	Heyns 1965
		11♂♂	3.62–4.66	49–63	4.0–4.9	78–118	0.8*	-	?	?	?	991*	?	44*	75–90	0–4		
		2♀♀	4.35–4.70	51–55	5.3–5.4	117–150	0.5–0.7	53–54	?	?	18	813–882	?	29–40	-	-	India	Khan et al. 1989
8	***silvaticum*** sp. n.	2♀♀	2.60, 2.56	29, ?	3.6, 3.8	83, 69	0.7	60, 58	17	12	13	720, 668	63, 60%	31, 37	-	-	Vietnam	Present paper
		4♂♂	2.09–2.58	28–33	3.4–4.2	67–94	0.6–0.7	-	17–18	11–12	12–13	597–684	58–64%	27–31	64–75	2–3		

* Calculated from original description. ** Abbreviations for columns: Lrd: Lip region diameter. Mural Odont. Vent: Mural odontostyle length at ventral side. Mural Odont. Dors.: Mural odontostyle length at dorsal side. Ph.exp.: Pharyngeal expansion length. Spicul.: Spicule length. Ve.sup.: Number of ventromedian supplements. Geog.dis.: Geographical distribution. ? This information is not available in the corresponding description.

Other valid species

Aporcelaimoides amazonicum (Siddiqi, 1995), comb. n.
  = *Sectonema amazonicum* Siddiqi, 1995
Aporcelaimoides brevistylum sp. n.
Aporcelaimoides californicum Heyns, 1965
  = *Sectonema californicum* (Heyns, 1965) Siddiqi, 1995
Aporcelaimoides haguei (Hunt, 1978), comb. n.
  = *Sectonema haguei* (Hunt, 1978) Andrássy, 2009
  Nygolaimium haguei Hunt, 1978
Aporcelaimoides minor sp. n.
Aporcelaimoides moderatum (Siddiqi, 1995), comb. n.
  = *Sectonema moderatum* Siddiqi, 1995
Aporcelaimoides silvaticum sp. n.

Remarks on some species

A. amazonicum: The nature of the stomatal protrusible structure, a mural odontostyle, supports its inclusion in *Aporcelaimoides* rather than in *Sectonema*.

A. haguei: Andrássy (2009) transferred this species to *Sectonema* from *Nygolaimium*, but the mural odontostyle that characterized this species justifies its transference to *Aporcelaimoides*.

A. moderatum: The general morphology of this species, very especially that of the stomatal protrusible structure, fits the updated concept of *Aporcelaimoides* and justifies its transference to this genus.

Key to identification of *Aporcelaimoides* species

1 Lip region nearly continuous with the adjacent body......... ***silvaticum* sp. n.**
– Lip region offset by constriction **2**
2 *Pars refringens vaginae* absent **3**
– *Pars refringens vaginae* present **5**
3 Larger (body 5.53 mm long) and more slender (*a* = 75) nematodes; neck comparatively shorter (*b* = 7.6); mural odontostyle aperture occupying one-half its length; male absent.. ***californicum***
– Smaller (body up to 4.75 mm long) and less slender (*a* up to 63) nematodes; neck comparatively longer (*b* up to 5.4); mural odontostyle aperture occupying 62–71% its length; male present.. **4**
4 Body 1.95–2.90 mm long and less slender (*a* = 25–35); shorter neck (663–767 µm long, *b* = 3.3–3.7); lip region 17–18 µm wide; comparatively longer tail (*c* = 49–76) .... ***brevistylum* sp. n.**

Altherr E (1965) La faune des sables submergés des rives du Rhin près de Krefeld. Nématodes. Gewässer und Abwässer, Düsseldorf 39/40: 80–101.

Andrássy I (1976) Evolution as a basis for the systematization of nematodes. Pitman Publishing, London, 288 pp.

Andrássy I (2009) Free-living nematodes of Hungary. III. Pedozoologica Hungarica n°5. Hungarian Natural History Museum, Budapest, Hungary, 608 pp.

Baermann G (1917) Eine einfache Methode zur Auffindung von *Ankylostomum* (Nematoden) Larven in Erdproben. Geneeskunding Tijdschrift voor Nederlandsch-Indië 57: 131–137.

Flegg JJM (1967) Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. Annals of Applied Biology 60: 429–437. doi: 10.1111/j.1744-7348.1967.tb04497.x

Gagarin VG, Gusakov VA (2012) *Mesodorylaimus dolichurus* sp. nov. (Nematoda, Dorylaimida) from freshwater body of Vietnam. Zoosystematica Rossica 21: 189–192.

Gagarin VG, Gusakov VA (2013a) Two species of dorylaimids (Nematoda) from waterbodies of Vietnam. Inland Water Biology 6: 176–183. doi: 10.1134/S199508291303005X

Gagarin VG, Gusakov VA (2013b) *Neotripyla vulgaris* gen. n., sp. n. and *Semitobrilus andrassyi* sp. n. (Nematoda, Triplonchida) from freshwater bodies of Vietnam. Zootaxa 3716: 565–576. doi: 10.11646/zootaxa.3716.4.4

Heyns J (1965) On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimoid nematodes. Entomology Memoirs, Department of Agricultural Technical Services, Republic of South Africa 10: 1–51.

Hunt DJ (1978) Bionomics of *Paractinolaimus vigor* Thorne, 1967 (Dorylaimida: Paractinolaimidae) with a description of *P. dominicus* n. sp. and *Nygolaimium haguei* n. sp. (Dorylaimida: Aporcelaimidae). Nematologica 23: 452–462. doi: 10.1163/187529277X00381

Jairajpuri MS, Ahmad W (1992) Dorylaimida. Free-living, predaceous and plant-parasitic nematodes. E.J. Brill, Leiden, 458 pp.

Khan TH, Jairajpuri MS, Ahmad W (1989) Description of some new and known species of dorylaim nematodes. Nematologica 35: 419–437. doi: 10.1163/002825989X00179

Loof PAA, Coomans A (1970) On the development and location of the oesophageal gland nuclei in Dorylaimina. Proceedings of the IX International Nematology Symposium (Warsaw, Poland, 1967), 79–161.

Nguyen TAD, Vu TT, Ahmad W, Peña-Santiago R (2011) New observations on *Thornedia opisthodelphis* (Jairajpuri, 1968) Siddiqi, 1982 (Dorylaimida, Leptonchidae) from Vietnam, with discussion on the identity of the genus. Journal of Nematode Morphology and Systematics 14: 63–69.

Nguyen TAD, Vu TT, Bonkowski M, Peña-Santiago R (2014) New data of three rare belondirid species (Nematoda, Dorylaimida, Belondiridae) from Vietnam, with the first record and description of the male of *Oxybelondira paraperplexa* Ahmad & Jairajpuri, 1979. Biodiversity Data Journal. doi: 10.3897/BDJ.2.e1156

Peña-Santiago R, Abolafia J, Álvarez-Ortega S (2014) New proposal for a detailed description of the dorylaim spicule (Nematoda: Dorylaimida). Nematology 16: 1091–1095. doi: 10.1163/15685411-00002834

Peña-Santiago R, Álvarez-Ortega S (2014a) Studies on the genus *Sectonema* Thorne, 1930 (Dorylaimida: Aporcelaimidae). Re-description of *S. ventrale*, the type species of the genus. Nematology 16: 1097–1104. doi: 10.1163/15685411-00002837

Peña-Santiago R, Álvarez-Ortega S (2014b) Re-description of three species of the genus *Sectonema* Thorne, 1930 (Nematoda: Dorylaimida: Aporcelaimidae) originally studied by E. Altherr. Zootaxa 3881: 63–74. doi: 10.11646/zootaxa.3881.1.5

Peña-Santiago R, Álvarez-Ortega S (2015) Description of *Sectonema septentrionale* sp. n. (Nematoda: Dorylaimida: Aporcelaimidae) from Northern Iberian Peninsula. Zootaxa 3947: 573–580. doi: 10.11646/zootaxa.3947.4.8

Siddiqi MR (1964) Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. Zeitschrift für Zoologische Systematik und Evolutionsforschung 2: 174–184. doi: 10.1111/j.1439-0469.1964.tb00720.x

Siddiqi MR (1984) *Sectonema anisonchum* sp. n., *S. mucrodens* sp. n. and *S. truxum* sp. n. (Nematoda: Dorylaimida) from Colombian rain forest. Indian Journal of Nematology 14: 84–88.

Siddiqi MR (1995) Nematodes of Tropical Rainforests. 5. Seven new genera and forty two new species of dorylaims. Afro-Asian Journal of Nematology 5: 72–109.

Thorne G (1930) Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. Journal of Agricultural Research USDA 41: 445–466.

Vu TT, Ciobanu M, Abolafia J, Peña-Santiago R (2010) Two remarkable new species of the genus *Crassolabium* Yeates, 1967 from Vietnam (Nematoda: Dorylaimida: Qudsianematidae). Journal of Natural History 44: 2049–2064. doi: 10.1080/00222933.2010.481055

Publication 3

Two atypical new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam

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Two atypical new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam

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Abstract. Two new species of the genus *Sectonema* from natural habitats of northern Vietnam are studied. This paper includes their descriptions, measurements, line illustrations, and light microscope (LM) and scanning electron microscope (SEM) pictures. *Sectonema tropicum* sp. nov. is characterized by a 2.56–3.24 mm long body, 19–21 µm broad lip region, odontostyle 20–21 µm long at its ventral side, 730–834 µm long neck, pharyngeal expansion occupying 52–59% of total neck length, uterus a simple tube-like structure 150–242 µm long or 1.2–2.5 times the body diameter, *pars refringens vaginae* present, $V = 48–52$, short (31–40 µm, $c = 70–91$, $c' = 0.5–0.6$) and rounded tail, 91–97 µm long spicules, and only one weakly developed ventromedian supplement. *Sectonema vietnamense* sp. nov. is characterized by its slender ($a = 33–49$) and 2.71–4.25 mm long body, 14–16 µm broad lip region, odontostyle 8–9 µm long at its ventral side, 716–918 µm long neck, pharyngeal expansion occupying 63–67% of total neck length, uterus simple and 209–242 µm long or 2.5–2.9 times the corresponding body diameter, *pars refringens vaginae* absent, $V = 54$, short (34–39 µm, $c = 70–115$, $c' = 0.6–0.8$) and rounded tail, 59–75 µm long spicules, and three or four irregularly spaced ventromedian supplements bearing hiatus. Both species are also characterized by their nearly continuous lip region, an atypical feature in this genus. Molecular analysis of *S. tropicum* sp. nov. confirms that *Sectonema* is a natural (monophyletic) taxon, very close to *Metaporcelaimus*.

Keywords. D2–D3 expansion segments of LSU ribosomal DNA, morphology, morphometrics, taxonomy, SEM.

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Introduction

This contribution presents partial results of two nematological projects that overlap in their objectives. On the one hand, it is the second in a series of papers devoted to studying the nematode diversity of aporcelaims (family Aporcelaimidae Heyns, 1965) from natural areas of Vietnam, the first one (Álvarez-Ortega *et al*. 2015) dealing with the representatives of the genus *Aporcelaimoides* Heyns, 1965. On the other hand, it is part of a general revision of the taxonomy and systematics of this dorylaimid group, in which the genera *Aporcelaimellus* Heyns, 1965, *Metaporcelaimus* Lordello, 1965 and *Sectonema* Thorne, 1930 have already received some attention (see for instance, and respectively, Álvarez-Ortega & Peña-Santiago 2013; Álvarez-Ortega *et al*. 2013a; Peña-Santiago & Álvarez-Ortega 2014a).

Interesting material belonging to the genus *Sectonema* was collected by the first author during several nematological surveys conducted in natural areas of Vietnam over the last years. The specimens are characterized by a nearly continuous lip region, indeed a rather atypical feature in *Sectonema*. Its detailed study revealed it to belong to two unknown forms, which are herein described.

Material and methods

Nematodes

Nematodes were collected from pristine areas in Northern Vietnam, extracted from soil samples using the methods by Baermann (1917) and Flegg (1967), relaxed and killed by heat, fixed in 4% formaldehyde, and processed to anhydrous glycerine following Siddiqi's (1964) technique. Finally, the specimens were mounted on permanent glass slides to allow handling and observation under LM.

Light microscopy

Specimens were measured using a light Olympus BH-2 microscope equipped with differential interference contrast (DIC). Morphometrics included de Man's indices and the usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof & Coomans (1970) and spicule terminology follows Peña-Santiago *et al*. (2014). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe® Photoshop® CS version 8.0.1. Drawings were made using a *camera lucida*.

Scanning electron microscopy

After examination and identification, a few of the preserved specimens were selected for observation under SEM following the protocol by Abolafia & Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope.

DNA extraction, PCR and sequencing

DNA was extracted from individuals using the proteinase K protocol. Nematode material was transferred to an 0.5 ml Eppendorf tube containing 18 µl of Worm Lysis Buffer (WLB) (50 mM KCL, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40, and 0.45% Tween 20) and 2 µl proteinase K (600 µg ml^{-1}) (Thermo Scientific). The tubes were incubated at 65°C (1 h) and then at 95°C (15 min). The PCR was performed in a 30 µl final volume containing 24.9 µl of sterile water, 0.6 µl of each PCR primer, 0.6 µl dNTP mixture, 0.3 µl *Taq* polyerase, 3 µl Buffer 10x Thermo Scientific Green and 1 µl of DNA extracted solution. The PCR amplification profile consisted of four min at 94°C, 35 cycles of 1 min at 94°C, 1.5 min at 55°C and 2 min at 72°C, followed by a final step of 10 min at 72°C. The primers used for amplification were D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) for amplification of D2–D3 region of 28S (Subbotin *et al*. 2006).

The PCR products were purified using Exo-SAP PCR cleanup containing 7 µl of PCR product, 0.15 µl Exonuclease I (Exo), 0.9 µl Shrimps Phosphatase Alkali (SAP) and 1.95 µl of sterile water. They were incubated at 37°C for 20 min and then heated up to 85°C for 15 min; then the purified solution was diluted ten times.

The sequencing reaction was performed with 1 µl of DNA purified template, 0.25 µl BigDye v3.1, 2.25 µl 5x BigDye sequencing buffer, 0.25 µl of one primer. The mixture was heated up for 10 s at 96°C, 5s at 55°C repeated for 32 cycles followed by 4 min at 60°C. The sequencing was performed at the Cologne Center for Genomics (CCG). The sequences obtained were submitted to the GenBank database under accession numbers KT868957 and KT868958.

Phylogenetic analyses

The newly obtained sequences were aligned with other forty two D2–D3 expansion segments of 28S rRNA gene sequences available in GenBank using ClustalX 1.83 (Thompson *et al.* 1997). Outgroup taxa were chosen according to the results of previously published data (Holterman *et al.* 2008; Álvarez-Ortega *et al.* 2013b). Sequence alignments were manually edited using GenDoc 2.6.002 (Nicholas *et al.* 1997). The sequence dataset was analysed with Bayesian inference (BI) and Maximum Likelihood (ML) using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and MEGA 6 (Tamura *et al.* 2013), respectively. The best fit model of DNA evolution for BI was obtained using the program MrModeltest 2.2 (Nylander 2002) with the Akaike Information Criterion in conjunction with PAUP* 4b10 (Swofford 2003). BI analysis under the GTR + G + I model was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 10^6 generations. ML analysis was implemented under the best-fitting evolutionary model (GTR + I + G), obtained using the program MEGA 6, and 1000 bootstrap replications. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. The trees were visualised with the program FigTree v1.4.0 and drawn with Adobe Acrobat XI Pro 11.0.1.

List of abbreviations

a = body length / greatest body diameter
b = body length / distance from anterior end to pharyngo-intestinal junction
c = body length / tail length
c' = tail length / tail diameter at anus or cloaca
DN = distance from body anterior end to the nucleus of pharyngeal dorsal gland expressed as percentage (%) of total neck length
DO = distance from body anterior end to the outlet of pharyngeal dorsal gland expressed as percentage (%) of total neck length
L = overall body length
n = number of specimens on which measurements are based
S_1N_1 = distance from body anterior end to the anterior nucleus of first pair of ventro-sublateral pharyngeal glands expressed as percentage (%) of total neck length
S_1N_2 = distance from body anterior end to the posterior nucleus of the first pair of ventro-sublateral pharyngeal glands expressed as percentage (%) of total neck length
S_2N = distance from body anterior end to the nuclei of the second pair of ventro-sublateral pharyngeal glands expressed as percentage (%) of total neck length
V = distance from body anterior end to vulva expressed as percentage (%) of the body length.

Results

Phylum Nematoda Cobb, 1932
Class Enoplea Inglis, 1983
Order Dorylaimida Pearse, 1942
Family Aporcelaimidae Heyns, 1965
Genus *Sectonema* Thorne, 1930

Sectonema tropicum sp. nov.
urn:lsid:zoobank.org:act:014CA9CB-57EE-43BB-BBFF-0C1A7EBD522D
Figs 1–4; Table 1

Diagnosis

The new species is characterized by a 2.56–3.24 mm long body, lip region hardly offset by very weak depression and 19–21 µm broad, odontostyle 20–21 µm long at its ventral side and 6.6–7.1 times as long as wide, 730–834 µm long neck, pharyngeal expansion 403–470 µm long or occupying 52–59% of total neck length, uterus a simple tube-like structure 150–242 µm long or 1.2–2.5 times the body diameter, *pars refringens vaginae* present, V = 48–52, short (32–39 µm, c = 70–88, c' = 0.5–0.6) and rounded female tail, male tail similar to that of female (31–40 µm, c = 73–91, c' = 0.5–0.6), 91–97 µm long spicules, and only one weakly developed ventromedian supplement.

Etymology

The specific epithet refers to the tropical area where the new species was collected.

Type material examined

Holotype

VIETNAM: ♀, in acceptable state of preservation, Northern Vietnam, Cao Bang Province, Pia Oac Natural Reserve, 22º 36'28'' N, 105º 52'15'' E, tropical evergreen forest soil associated with *Machilus* sp. and *Dimocarpus* sp., deposited in the nematode collection of the University of Jaén, Spain.

Paratypes

VIETNAM: 4 ♀♀, 3 ♂♂, in acceptable state of preservation, same data as holotype; 1 ♀, 1 ♂, in acceptable state of preservation, same locality, deposited in the nematode collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam; 1 ♂, same locality, used for SEM.

Description

Adult

Moderately slender to slender nematodes of medium to big size, 2.56–3.24 mm long. Body cylindrical, distinctly tapering towards the anterior end, less so towards the posterior end as the caudal region is short and rounded. Habitus curved ventrad after fixation, especially in posterior body region, C-, G- or spiral-shaped. Cuticle 3.0–4.5 µm thick at anterior region, 5–6 µm in mid-body and 8–10 µm on tail; consisting of three layers, especially distinguishable at caudal region: thinner outer layer bearing very fine transverse striation across the entire body, thicker intermediate layer with radial striation, and thin inner layer. Lateral chords 13–21 µm wide at mid-body, occupying about one-sixth (13–18%) of mid-body diameter. Two ventral and two dorsal body pores often present at level of odontostyle-odontophore. Lip region hardly offset from the adjacent body by weak, but perceptible depression, 2.8–3.0 times wider than high and up to one-fourth (17–25%) of body diameter at neck base; lips (under SEM) mostly amalgamated, but their perioral part distinctly separated by the existence of six radial, interlabial, deep incisures delimiting six perceptible liplets; button-like labial papillae, the inner ones located at the margin of the oral field and surrounded by two or three concentric annuli, whereas the outer papillae, located

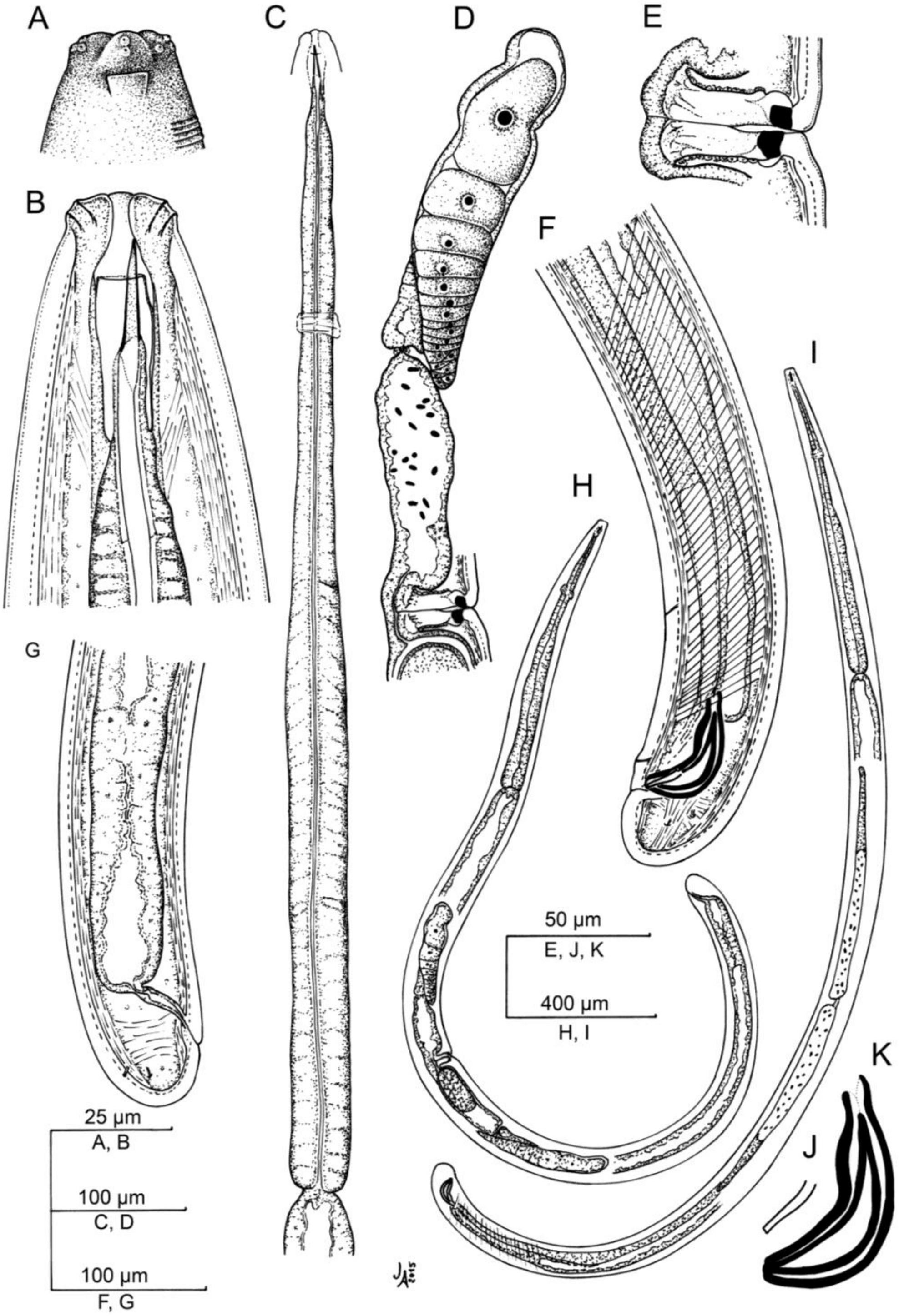

Fig. 1. *Sectonema tropicum* sp. nov. (Line). **A**. Lip region in lateral surface view. **B**. Anterior region in mid-optical level, lateral view. **C**. Neck region. **D**. ♀, anterior genital branch. **E**. Vagina. **F**. ♂, posterior body region. **G**. ♀, posterior body region. **H**. ♀, entire. **I**. ♂, entire. **J**. Lateral guiding piece. **K**. Spicule.

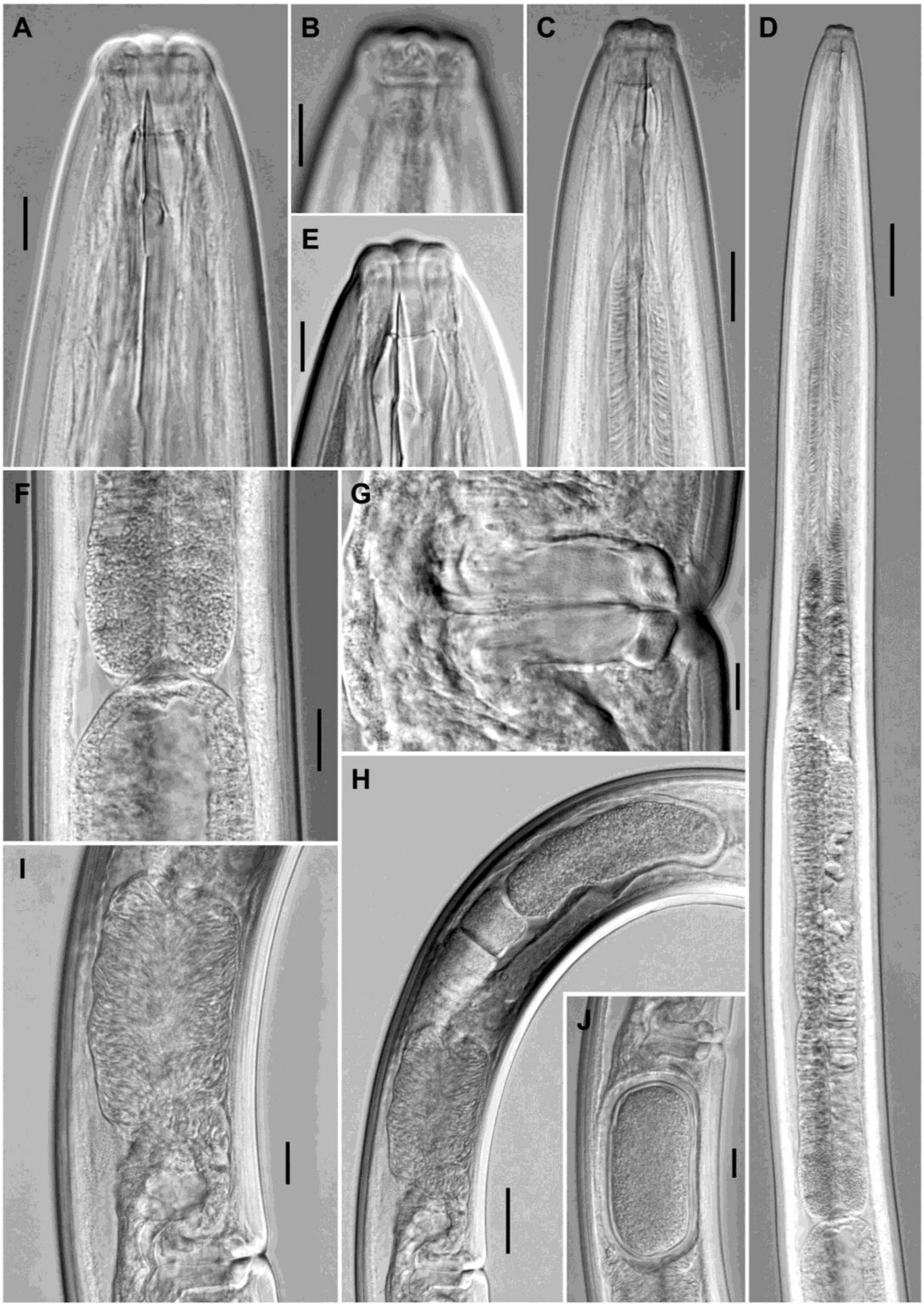

Fig. 2. *Sectonema tropicum* sp. nov. (LM). **A**, **C**, **E**. Anterior region in lateral, median view. **B**. Lip region in lateral, surface view. **D**. Neck region. **F**. Pharyngo-intestinal junction. **G**. Vagina. **H**. ♀, anterior genital branch. **I**. Vagina and anterior uterus containing sperm cells inside. **J**. Uterine egg. Scale bars: A–B, E, G = 10 µm; C, F, I–J = 20 µm; D, H = 50 µm.

a little behind the inner papillae, are surrounded by only one annulus; cephalic papillae pore- rather than button-like, also surrounded by only one ring-like annulus; oral aperture a dorso-ventral, slightly hexagonal orifice, the lip region hence showing a bi-radial symmetry. Amphid fovea cup-shaped, its opening occupying 9–10 µm or less than one-half (43–49%) of lip region diameter. Cheilostom nearly cylindrical, without any differentiation. Stomatal protruding structure apparently a reduced odontostyle 6.6–7.1 times longer than wide (see: Remarks), its ventral side 1.0–1.1 times longer than lip region diameter and 0.65–0.83% of body length. Guiding ring simple, plicate, at 0.8–0.9 lip region diameters from the anterior end. Odontophore linear, rod-like, 1.7–1.9 times the odontostyle length, somewhat irregular at its base and with (in lateral view) the ventral side slightly longer than the dorsal one (Fig. 2A). Anterior region of pharynx enlarging very gradually; basal expansion 7.1–10.7 times longer than wide, 3.6–5.6 times as long as body diameter, and occupying 52–59% of total neck length; gland nuclei

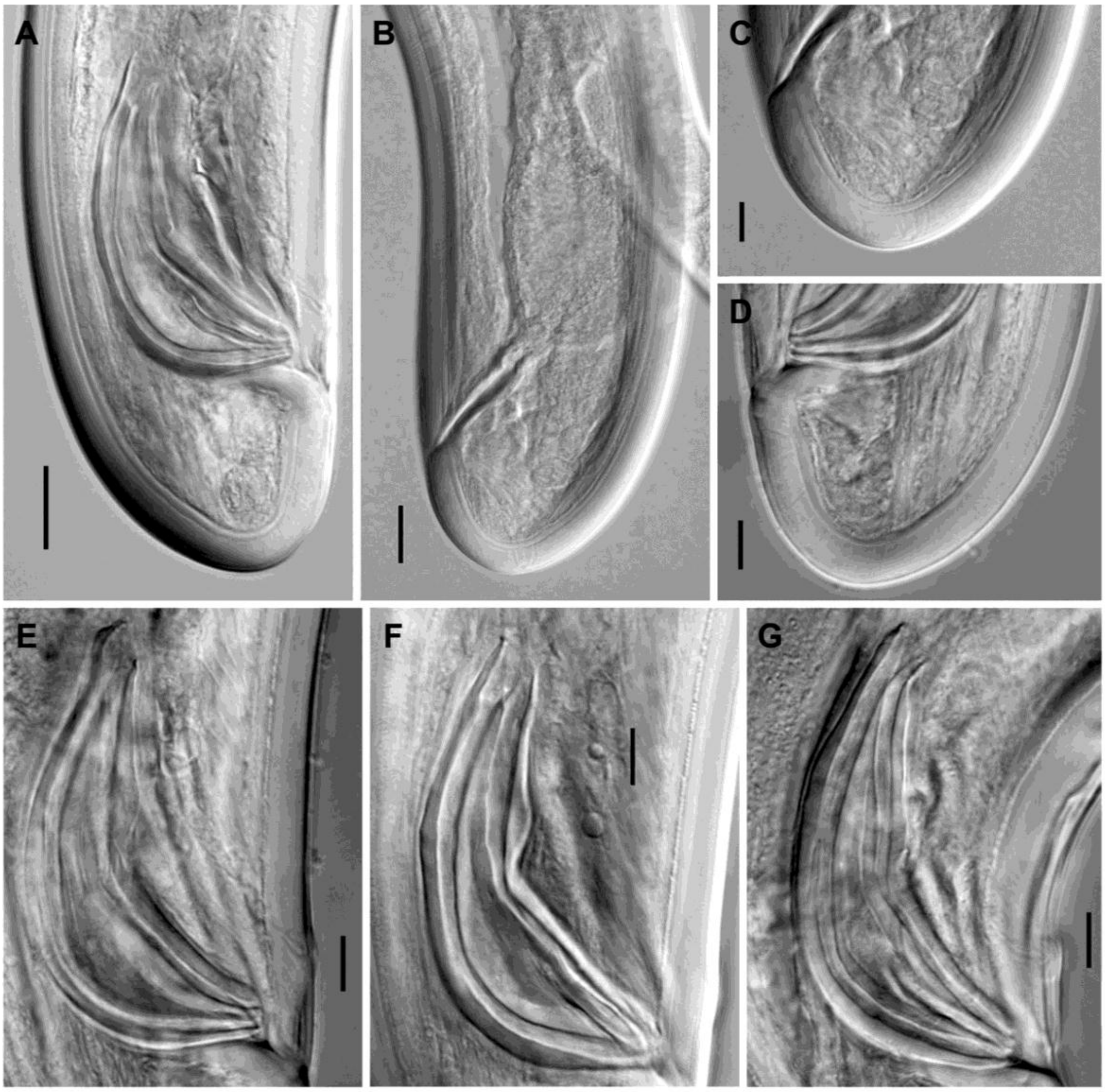

Fig. 3. *Sectonema tropicum* sp. nov. (LM). **A**. ♂, posterior body region. **B**. ♀, posterior body region. **C**. ♀, caudal region. **D**. ♂, caudal region. **E–G**. Spicules, E–F also showing the lateral guiding piece. Scale bars: A–B = 20 µm; C–G = 10 µm.

often obscure, located as follows: DN = 60–62 (n = 4); S_1N_1 = 75 (n = 2); S_1N_2 = obscure; S_2N = obscure. Nerve ring located at 182–216 µm from anterior end or 25–26% of total neck length. Cardia rounded conoid, 13–17 × 13–17 µm; a weak ring-like structure is present surrounding its junction to pharyngeal base.

Female

Genital system didelphic-amphidelphic, with almost equally and well developed branches, the anterior 320–442 µm or 12–15% of body length and the posterior 348, 440 µm (n = 2) or 14, 16% of body length (447, 509 µm or 15, 17% bearing one uterine egg inside). Reflexed ovaries well developed, often surpassing the sphincter level, the anterior 182–319 µm, the posterior 207–380 µm long; oocytes arranged first in two or more rows, then in a single row. Oviduct 124–174 µm long or 1.1–1.8 times the

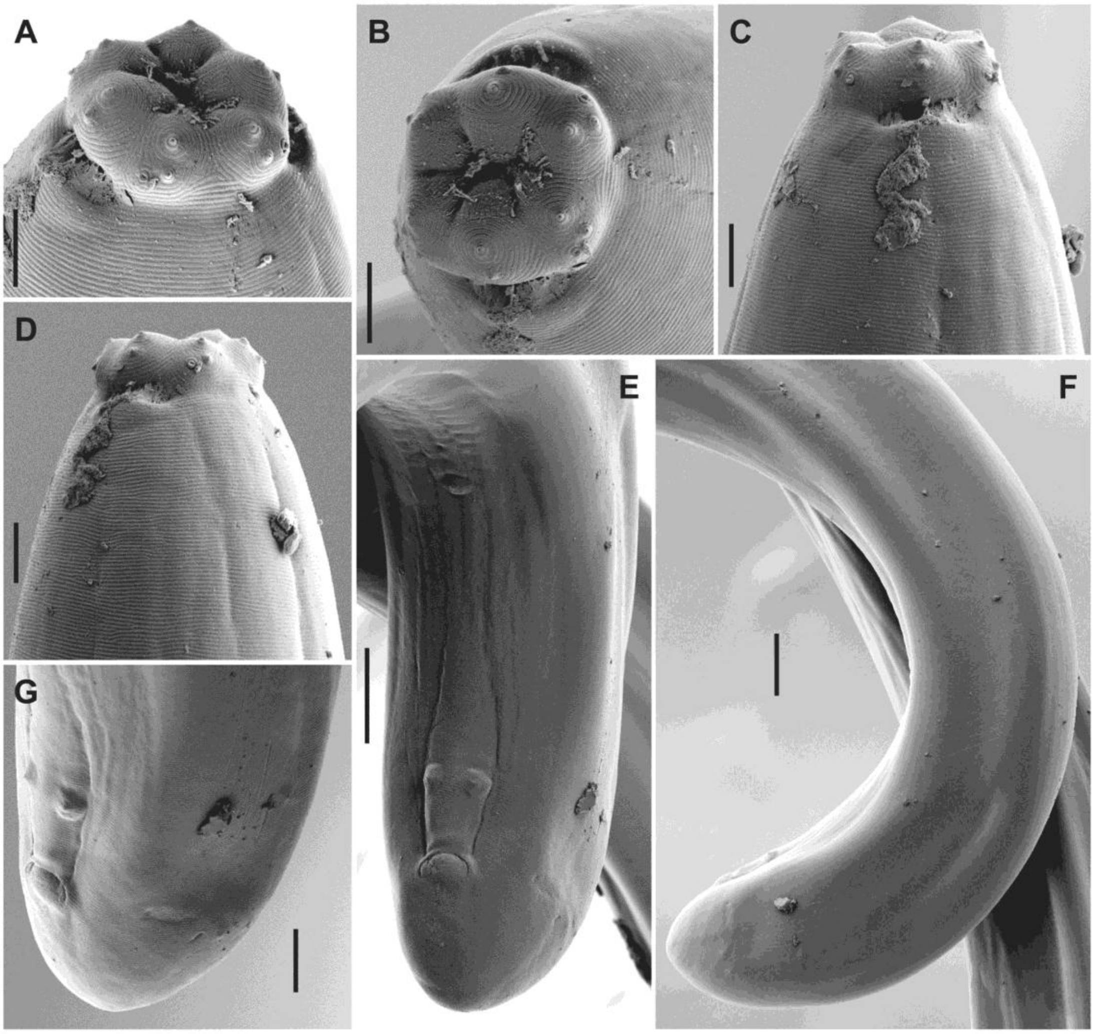

Fig. 4. *Sectonema tropicum* sp. nov. (SEM, ♂). **A**. Lip region in ventral view. **B**. Lip region in frontal view. **C**. Lip region in lateral view. **D**. Lip region in sublateral view. **E**. Posterior body region in ventral view. **F**. Posterior body region in lateral view. **G**. Caudal region in sublateral view. Scale bars: A–D = 5 µm; E–F = 20 µm; G = 10 µm

corresponding body diameter, and consisting of a slender part made of prismatic cells and a moderately developed *pars dilatata,* with visible lumen and often containing sperm cells inside. Oviduct-uterus junction marked by a sphincter. Uterus a simple, tube-like structure 150–190 µm long or 1.2–1.7 times the corresponding body diameter [223, 242 µm (n = 2) long or 2.2, 2.5 times the corresponding body diameter, with one uterine egg inside; and 231, 240 µm (n = 1) long, with two uterine eggs], always containing abundant sperm cells inside. Vagina extending inwards 52–57 µm or one-half to four-sevenths (48–57%) of body diameter: *pars proximalis* 33–43 × 23–28 µm, with somewhat sigmoid walls surrounded by weak musculature; *pars refringens* with two trapezoidal pieces measuring (in optical section) 13–16 × 7–9 µm and with a combined width of 24–28 µm; *pars distalis* 5.5–7.0 µm long. Vulva a nearly equatorial transverse slit. Prerectum 2.0–2.4, rectum 0.7–1.0 anal body diameters long. Tail short and rounded; two pairs of caudal pores, sublateral and close together.

Male

Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 18–21 µm from cloacal aperture, one weakly developed ventromedian supplement, lying out the range of retracted spicules and located at 80–131 µm from the ad-cloacal pair. Spicules distinctly robust and massive, especially in its posterior half, 3.6–4.2 times its maximum width, 1.4–1.6 times the body diameter at level of the cloacal aperture: dorsal contour regularly convex, ventral contour slightly concave, with distinct hump and hollow; curvature 121–124°; head occupying 11–16% of spicule total length, its dorsal side visibly curved at its anterior end and longer than the ventral one, which is shorter and almost straight; median piece 5.4–6.2 times longer than wide, occupying 51–59% of spicule maximum width, reaching the posterior tip; posterior end 7–8 µm wide. Lateral guiding pieces 23–29 µm long, 5.3–7.1 times longer than wide. Prerectum 3.2–4.6, cloaca 0.9–1.3 times the corresponding body width long. Cloacal aperture, as seen under SEM, a curved anteriad, transverse slit. Tail similar to that of female; caudal pores two pairs, one lateral, another subdorsal.

Relationships

In having a long stomatal protruding structure (20–21 µm at its ventral side) and short (*c'* up to 1.0) and rounded tail, *S. tropicum* sp. nov. is morphologically very similar to *S. demani* Altherr, 1965, *S. heynsi* Altherr, 1968, *S. rotundicauda* Goodey, 1951, *S. septentrionale* Peña-Santiago & Álvarez-Ortega, 2015 and *S. sica* Clark, 1964, but it can be distinguished from all of them in its smaller general size (body length 2.56–3.24 mm, neck 730–834 µm long vs more than 5 mm, more than 1000 µm long, respectively), nearly continuous lip region (vs offset by a more or less deep constriction) and stomatal protruding structure of different nature (a reduced odontostyle vs a mural tooth; see Remarks). Besides, the new species differs from *S. demani* (see recent re-description by Peña-Santiago & Álvarez-Ortega 2014b) in its less slender (*a* = 21–36 vs *a* = 48–56) body, narrower (19–21 vs 27–28 µm broad) lip region, shorter pharyngeal expansion (occupying 52–59 vs 68–69% of total neck length), shorter female tail (32–39 vs 51–55 µm, *c'* = 0.5–0.6 vs *c'* = 0.7–0.8), and male present (vs absent). It differs from *S. heynsi* (see recent re-description by Peña-Santiago & Álvarez-Ortega 2014b) in its narrower (19–21 vs 28 µm broad) lip region, circumoral area lacking (vs bearing) cilia- or setae-like structures, shorter pharyngeal expansion (occupying 52–59 vs 68% of total neck length), shorter (32–39 vs 54 µm, *c'* = 0.5–0.6 vs *c'* = 0.7) female tail, and male present (vs absent). It differs from *S. rotundicauda* in its less slender (*a* = 21–36 vs *a* = 80) body, narrower (19–21 vs about 28 µm broad) lip region, shorter (31–40 vs 64 µm) male tail, fewer (one vs four) ventromedian supplements, and shorter (91–97 vs about 155 µm) spicules. It differs from *S. septentrionale* in its less slender (*a* = 21–36 vs *a* = 47–64) body, narrower (19–21 vs 25–29 µm broad) lip region, shorter (occupying 52–59 vs 60–73% of total neck length) pharyngeal expansion, uterus simple (vs tripartite) and shorter (150–242 µm or 1.2–2.5 times the body diameter vs 370–493 µm or 2.9–4.3 times the corresponding body diameter), shorter (31–40 vs 40–62 µm, *c'* = 0.5–0.6 vs *c'* = 0.6–0.8) tail, fewer (one vs 5–11) ventromedian supplements, and shorter (91–97 vs 100–145 µm) spicules. Finally, it differs from *S. sica* in its narrower (19–21 vs about 24 µm

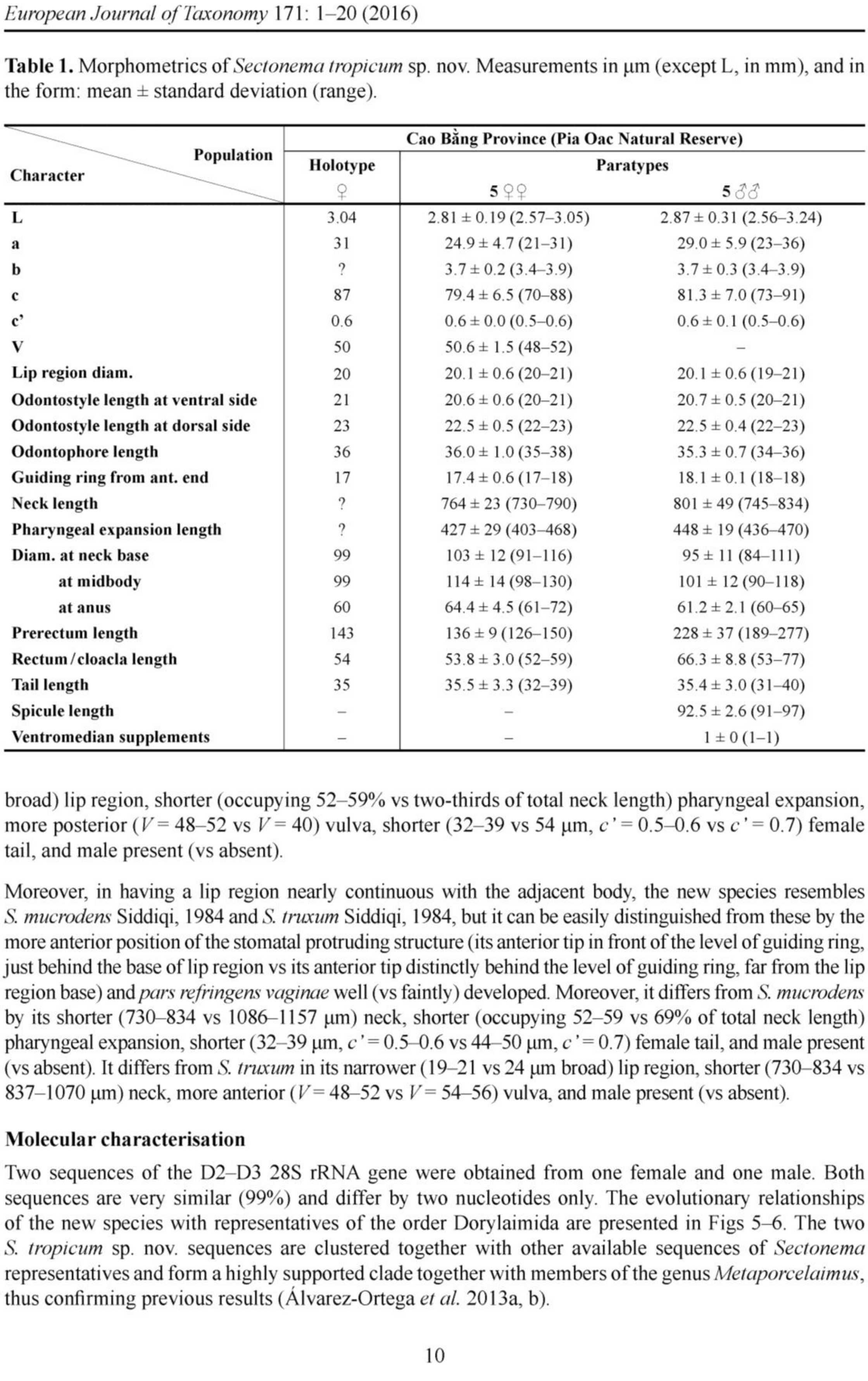

Table 1. Morphometrics of *Sectonema tropicum* sp. nov. Measurements in μm (except L, in mm), and in the form: mean ± standard deviation (range).

Character / Population	Cao Bằng Province (Pia Oac Natural Reserve)		
	Holotype	Paratypes	
	♀	5 ♀♀	5 ♂♂
L	3.04	2.81 ± 0.19 (2.57–3.05)	2.87 ± 0.31 (2.56–3.24)
a	31	24.9 ± 4.7 (21–31)	29.0 ± 5.9 (23–36)
b	?	3.7 ± 0.2 (3.4–3.9)	3.7 ± 0.3 (3.4–3.9)
c	87	79.4 ± 6.5 (70–88)	81.3 ± 7.0 (73–91)
c'	0.6	0.6 ± 0.0 (0.5–0.6)	0.6 ± 0.1 (0.5–0.6)
V	50	50.6 ± 1.5 (48–52)	–
Lip region diam.	20	20.1 ± 0.6 (20–21)	20.1 ± 0.6 (19–21)
Odontostyle length at ventral side	21	20.6 ± 0.6 (20–21)	20.7 ± 0.5 (20–21)
Odontostyle length at dorsal side	23	22.5 ± 0.5 (22–23)	22.5 ± 0.4 (22–23)
Odontophore length	36	36.0 ± 1.0 (35–38)	35.3 ± 0.7 (34–36)
Guiding ring from ant. end	17	17.4 ± 0.6 (17–18)	18.1 ± 0.1 (18–18)
Neck length	?	764 ± 23 (730–790)	801 ± 49 (745–834)
Pharyngeal expansion length	?	427 ± 29 (403–468)	448 ± 19 (436–470)
Diam. at neck base	99	103 ± 12 (91–116)	95 ± 11 (84–111)
at midbody	99	114 ± 14 (98–130)	101 ± 12 (90–118)
at anus	60	64.4 ± 4.5 (61–72)	61.2 ± 2.1 (60–65)
Prerectum length	143	136 ± 9 (126–150)	228 ± 37 (189–277)
Rectum / cloacla length	54	53.8 ± 3.0 (52–59)	66.3 ± 8.8 (53–77)
Tail length	35	35.5 ± 3.3 (32–39)	35.4 ± 3.0 (31–40)
Spicule length	–	–	92.5 ± 2.6 (91–97)
Ventromedian supplements	–	–	1 ± 0 (1–1)

broad) lip region, shorter (occupying 52–59% vs two-thirds of total neck length) pharyngeal expansion, more posterior (*V* = 48–52 vs *V* = 40) vulva, shorter (32–39 vs 54 μm, *c'* = 0.5–0.6 vs *c'* = 0.7) female tail, and male present (vs absent).

Moreover, in having a lip region nearly continuous with the adjacent body, the new species resembles *S. mucrodens* Siddiqi, 1984 and *S. truxum* Siddiqi, 1984, but it can be easily distinguished from these by the more anterior position of the stomatal protruding structure (its anterior tip in front of the level of guiding ring, just behind the base of lip region vs its anterior tip distinctly behind the level of guiding ring, far from the lip region base) and *pars refringens vaginae* well (vs faintly) developed. Moreover, it differs from *S. mucrodens* by its shorter (730–834 vs 1086–1157 μm) neck, shorter (occupying 52–59 vs 69% of total neck length) pharyngeal expansion, shorter (32–39 μm, *c'* = 0.5–0.6 vs 44–50 μm, *c'* = 0.7) female tail, and male present (vs absent). It differs from *S. truxum* in its narrower (19–21 vs 24 μm broad) lip region, shorter (730–834 vs 837–1070 μm) neck, more anterior (*V* = 48–52 vs *V* = 54–56) vulva, and male present (vs absent).

Molecular characterisation

Two sequences of the D2–D3 28S rRNA gene were obtained from one female and one male. Both sequences are very similar (99%) and differ by two nucleotides only. The evolutionary relationships of the new species with representatives of the order Dorylaimida are presented in Figs 5–6. The two *S. tropicum* sp. nov. sequences are clustered together with other available sequences of *Sectonema* representatives and form a highly supported clade together with members of the genus *Metaporcelaimus*, thus confirming previous results (Álvarez-Ortega *et al.* 2013a, b).

Remarks

The nature of the stomatal protruding structure in the new species is difficult to interpret and deserves further explanation. The specimens examined bear a reduced odontostyle (comparable to that described in the type species *S. ventrale* Thone, 1930; see recent description by Peña-Santiago & Álvarez-Ortega

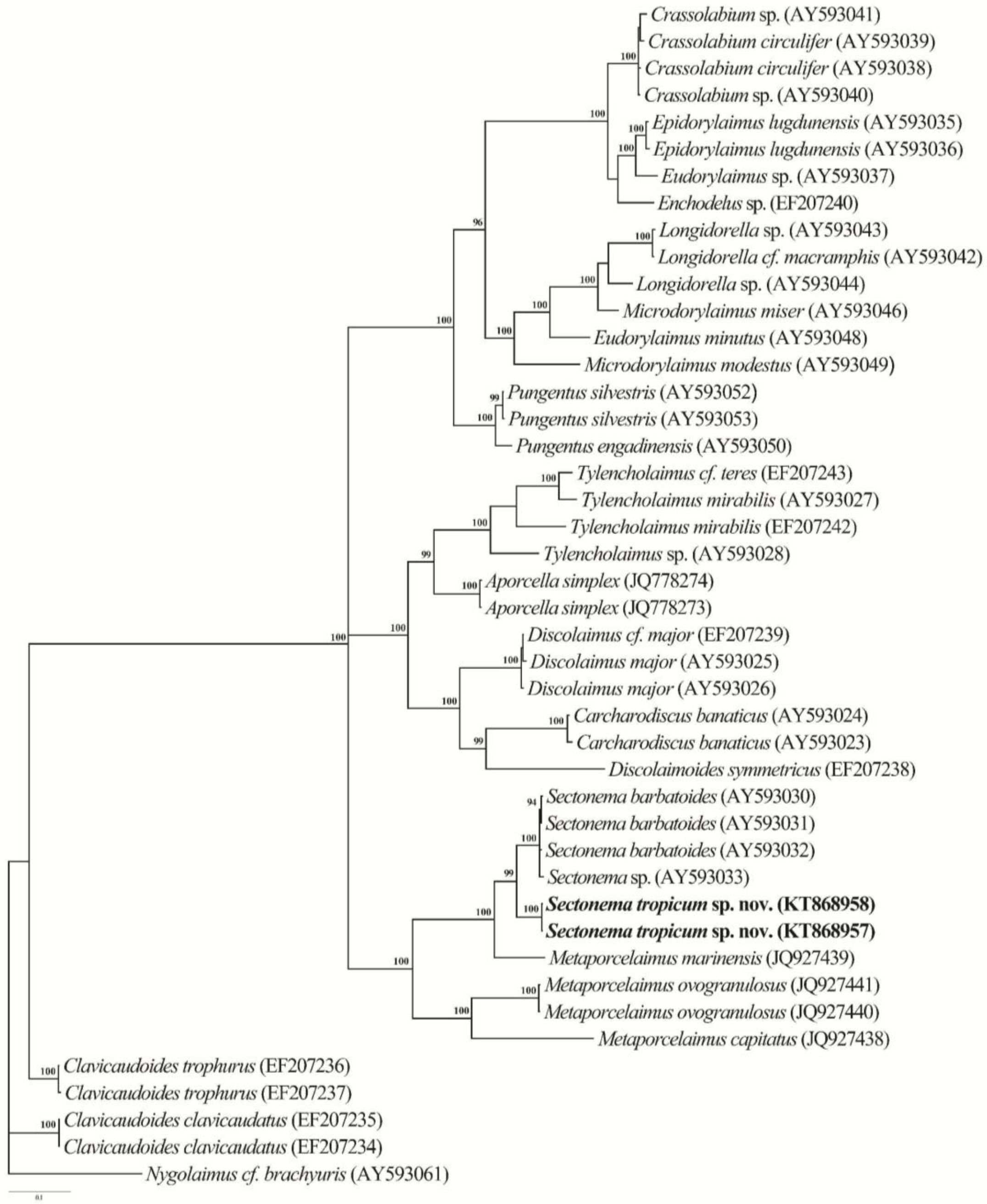

Fig. 5. Bayesian 50% majority rule consensus trees as inferred from D2–D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Posterior probabilities are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

2014a) rather than a mural tooth as the base of its dorsal side is visibly sclerotized and seems to join the dorsal stomatal wall (vs dorsal side not scletorized at the base, somewhat sigmoid and not joining the dorsal stomatal wall). Nonetheless, some doubt persists in this matter.

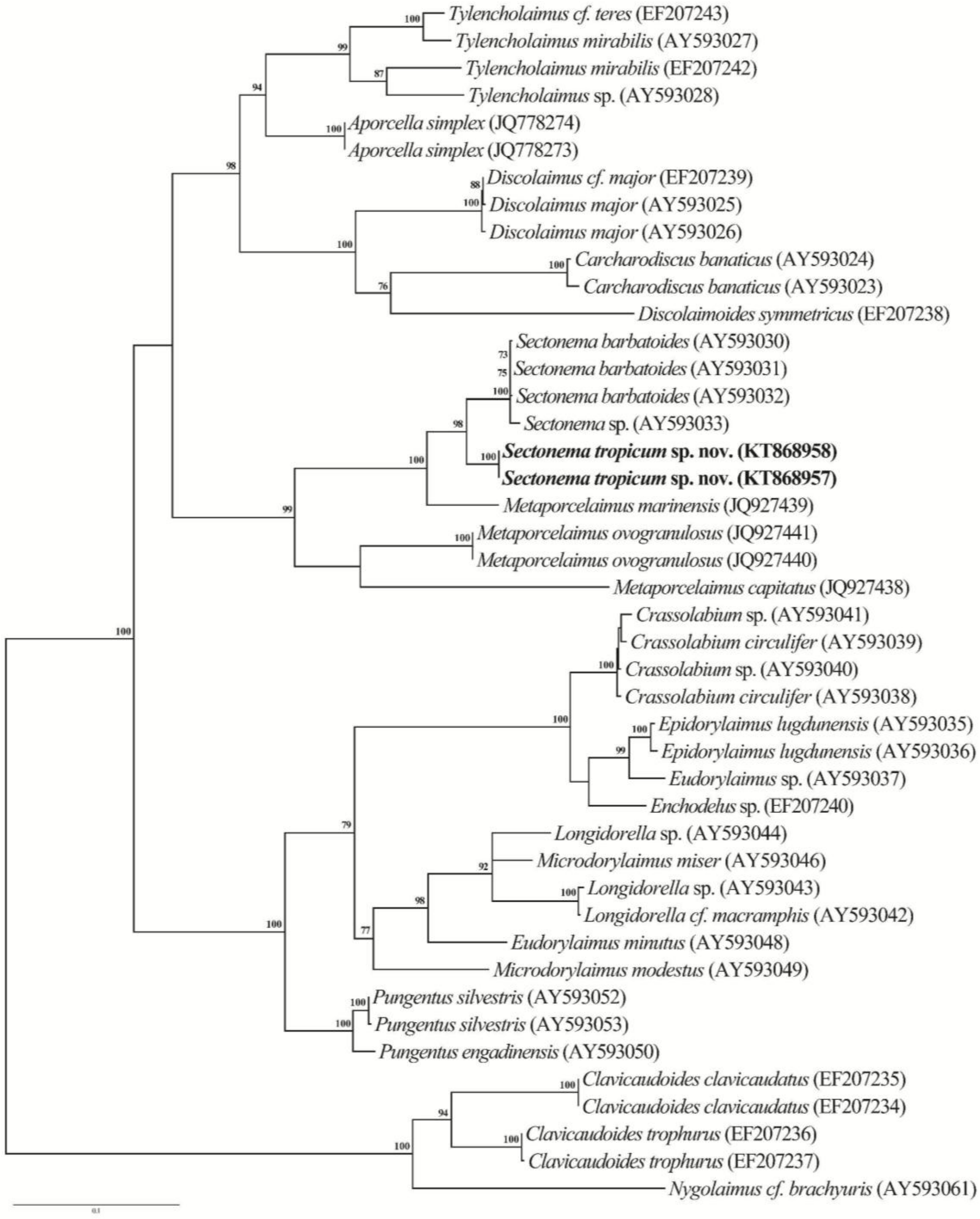

Fig. 6. Maximum Likelihood tree as inferred from D2–D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Bootstrap values are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

Sectonema vietnamense sp. nov.

urn:lsid:zoobank.org:act:AF58451E-D7A7-4E49-8A18-9F3998EF4A87

Figs 7–9, Table 2

Diagnosis

The new species is characterized by a slender (a = 33–49) 2.71–4.25 mm long body, lip region offset by depression and 14–16 µm broad, odontostyle 8–9 µm long at its ventral side, 716–918 µm long neck, pharyngeal expansion 463–618 µm long or 63–67% of total neck length, uterus simple and 209–242 µm long or 2.5–2.9 times the corresponding body diameter, *pars refringens vaginae* absent, V = 54, short (36 µm, c = 105, c' = 0.7) and rounded tail in female and slightly more conoid (34–39 µm, c = 70–115, c' = 0.6–0.8) in male, 59–75 µm long spicules, and three or four irregularly spaced ventromedian supplements bearing hiatus.

Etymology

The specific epithet refers to the geographical origin of the species.

Type material examined

Holotype

VIETNAM: ♀, in good state of preservation, Northern Vietnam, Cuc Phuong National Park, pristine tropical forest, deposited in the nematode collection of the University of Jaén, Spain.

Paratypes

VIETNAM: 4 ♂♂, 3 juvs, in good state of preservation, same data as holotype; 1 ♂, in good state of preservation, same locality, deposited in the nematode collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam; 1♂, 1 juv., same locality, used for SEM.

Other material examined

VIETNAM: 2 ♂♂, in acceptable state of preservation, Northern Vietnam, Thái Bình province, intensively cultured field, deposited in the nematode collection of the University of Jaén, Spain.

Description (type population)

Adult

Very slender nematodes of big size, 3.13–4.25 mm long. Body cylindrical, distinctly tapering towards the anterior end, less so towards the posterior one as the caudal region is rounded. Habitus regularly curved ventrad after fixation, to a more or less open C, occasionally more curved at posterior body region. Cuticle 2.0–2.5 µm thick at anterior region, 3.0–4.5 µm in mid-body and 8–10 µm on tail; consisting of three layers especially distinguishable at caudal region: thinner outer layer bearing very fine transverse striation through the entire body, thicker intermediate layer with radial striation, and thin inner layer. Lateral chords 6–12 µm wide at mid-body, occupying up to one-sixth (8–15%) of mid-body diameter. Two ventral and two dorsal body pores often present at level of stoma, and four distinct lateral pores 6–20 µm apart visible behind the amphid fovea. Lip region nearly continuous (a shallow depression is, however, often perceptible), weakly angular, visibly narrower than adjacent body, 2.1–2.3 times wider than high and about one-fifth (18–23%) of body diameter at neck base; it appears (under SEM) marked by radial (oral field) or longitudinal (remaining lip region) incisures, with amalgamated lips and protruding, pore-like labial and cephalic papillae, all of them surrounded by a ring-like annulus, and the inner labial papillae visibly larger than the outer ones; oral field perceptibly hexagonal, oral aperture a dorso-ventral slit, the lip region hence showing a bi-radial symmetry. Cup-shaped amphid fovea, its opening at level of cephalic depression and occupying 10–11 µm or 68–72% of lip region diameter. Nearly cylindrical cheilostom, without differentiation. Odontostyle comparatively

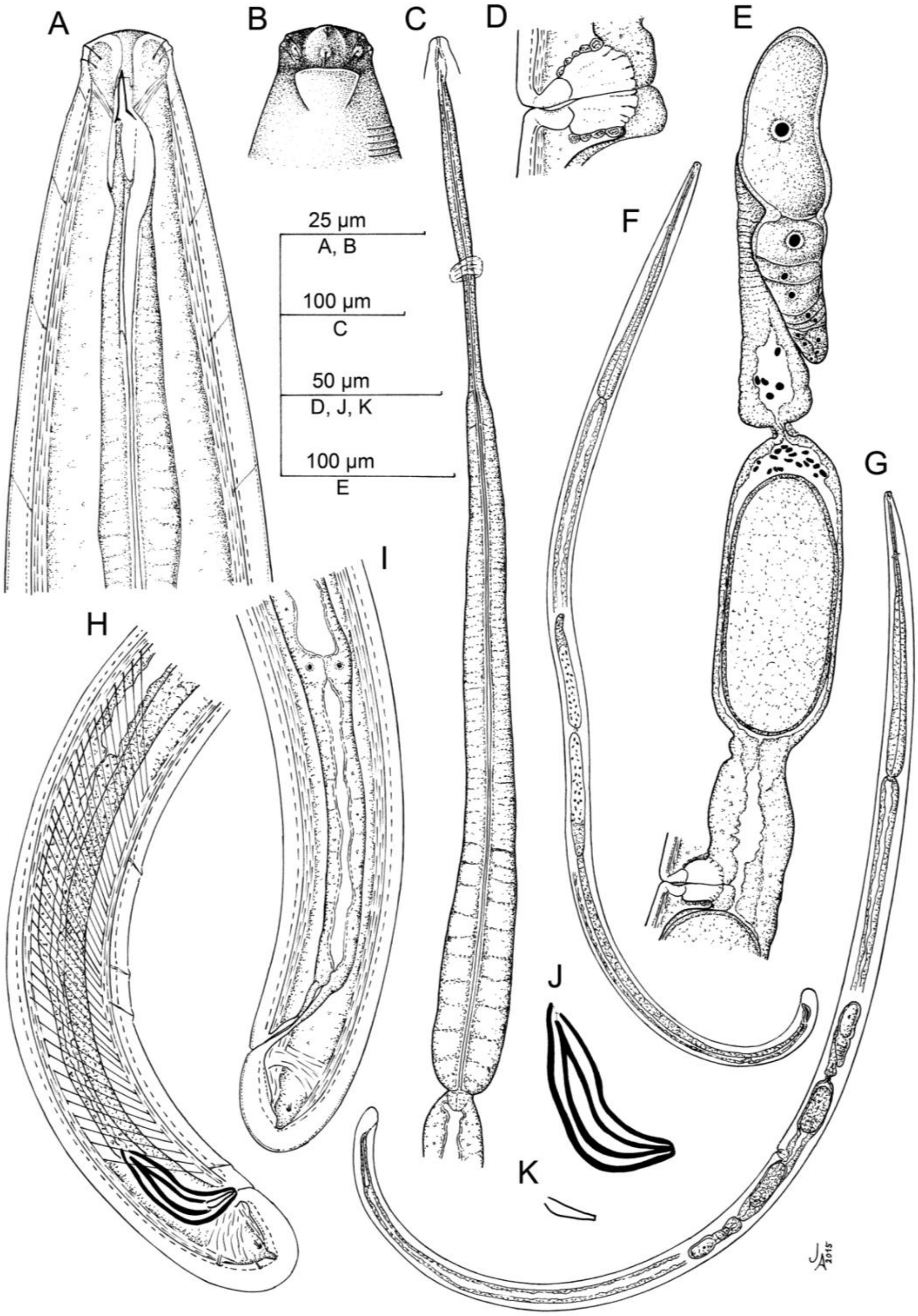

Fig. 7. *Sectonema vietnamense* sp. nov. (Line). **A**. Anterior region in median, lateral view. **B**. Lip region in surface, lateral view. **C**. Neck region. **D**. Vagina. **E**. ♀, anterior genital branch. **F**. ♂, entire. **G**. ♀, entire. **H**. ♂, posterior body region. **I**. ♀, posterior body region. **J**. Spicule. **K**. Lateral guiding piece.

short and occupying the whole stomatal lumen, its ventral side 0.5–0.6 times longer than lip region diameter and 0.20–0.29% of body length. Guiding ring simple, somewhat plicate, at 0.5 times the lip region diameter from anterior end. Odontophore linear, rod-like, 3.7–4.2 times the odontostyle length. Tripartite pharynx, consisting of an anteriorly thickened section behind the odontophore base, a perceptible narrower intermediate section with the nerve ring surrounding it, which enlarges very gradually into the basal expansion 9.9–15.0 times longer than wide, 6.7–7.3 times longer than body

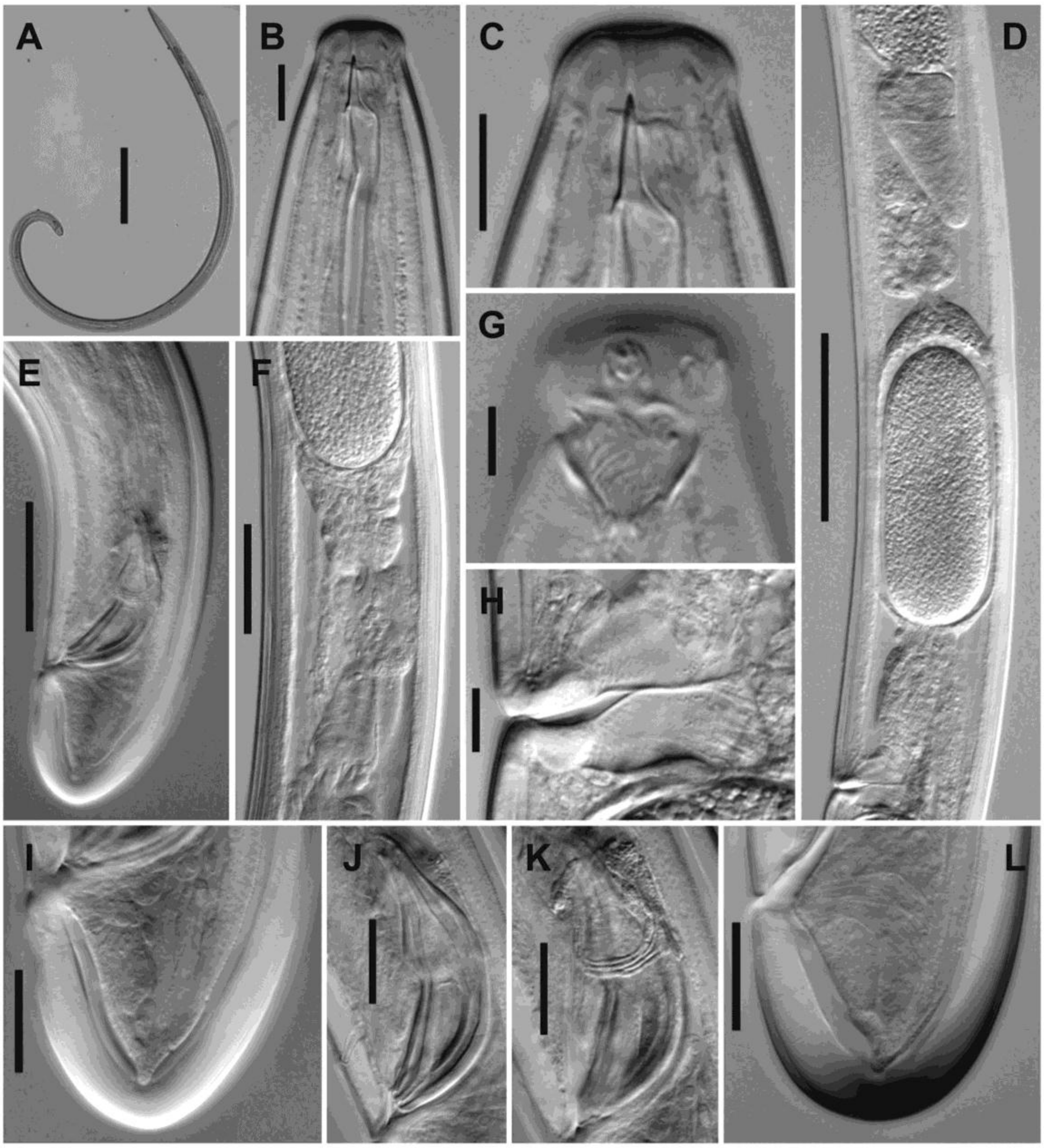

Fig. 8. *Sectonema vietnamense* sp. nov. (LM, type population). **A**. ♂, entire. **B–C**. Anterior region in lateral, median view. **D**. ♀, anterior genital branch. **E**. ♂, posterior body region. **F**. Oviduct-uterus junction. **G**. Lip region in lateral, surface view. **H**. Vagina. **I**. ♂, caudal region. **J–K**. Spicules. **L**. ♀, caudal region. Scale bars: A = 500 μm; B–C, H = 10 μm; D = 100 μm; E–F = 50 μm; G = 5 μm; I–L = 20 μm.

diameter and occupying 63–67% of total neck length; gland nuclei mostly obscure, only S_2N are visible, at 77–78% (n = 3) of total neck length. Nerve ring located at 147–178 µm from anterior end or 19–22% of total neck length. Cardia rounded conoid, 15–19 × 12–17 µm; a ring-like structure is present surrounding its junction to pharyngeal base.

Female

Genital system didelphic-amphidelphic, with almost equally and well developed branches, the anterior 473 µm long or 13% of body length and the posterior 387 µm long or 10% of body length, each branch bearing one uterine egg. Moderately sized ovaries, not surpassing the sphincter level, the anterior 191 µm, the posterior 141 µm long; oocytes arranged first in two or more rows, then in a single row. Oviduct 117, 142 µm long or 1.4, 1.7 times the corresponding body diameter, and consisting of a slender part made of prismatic cells and a well developed *pars dilatata* with wide lumen and sperm cells inside. Oviduct-uterus junction marked by a sphincter. Uterus a simple, tube-like structure 209, 242 µm long or 2.5, 2.9 times the corresponding body diameter, containing abundant sperm cells and one uterine egg. Vagina extending inwards 36 µm or about three-sevenths (44%) of body diameter: *pars proximalis* 23 × 28 µm, somewhat sigmoid walls and surrounded by weak musculature; *pars refringens* absent; *pars*

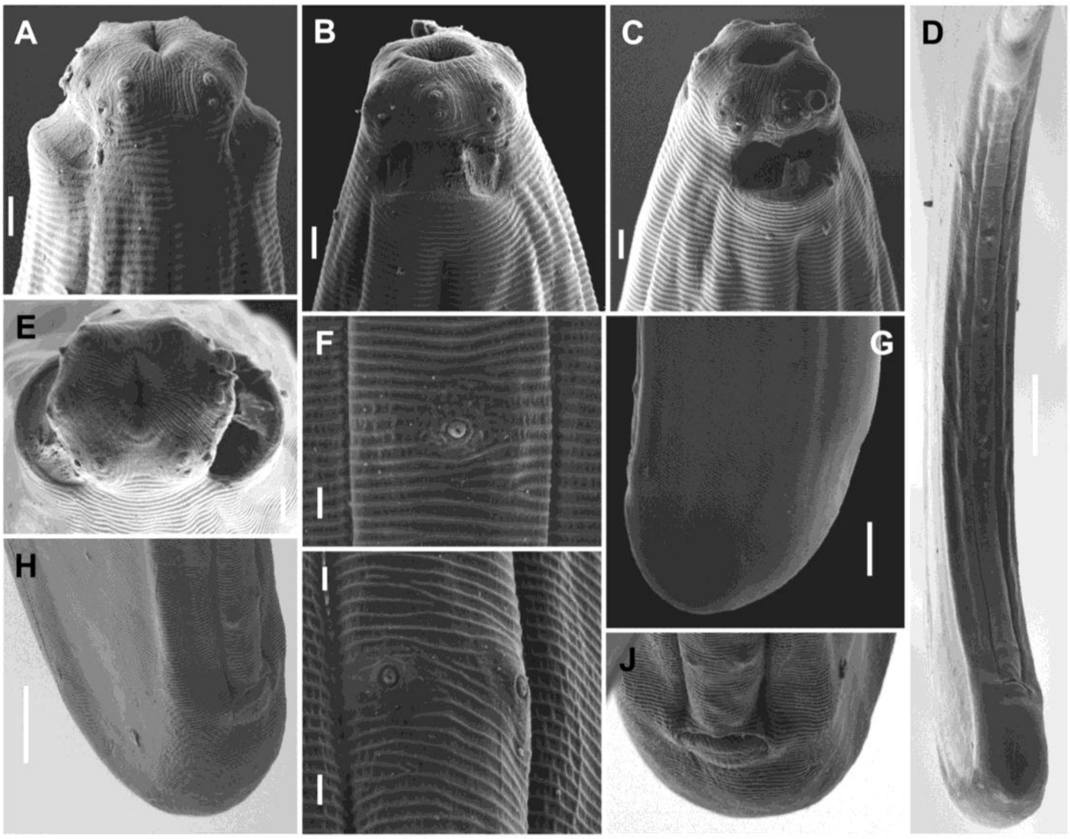

Fig. 9. *Sectonema vietnamense* sp. nov. (SEM, A = juvenile and B–J = ♂). **A**. Lip region in ventral view. **B–C**. Lip region in lateral view. **D**. Posterior body region in ventral view. **E**. Lip region in frontal view. **F**. Detail of a ventromedian supplement. **G**. Caudal region in lateral view. **H**. Caudal region in subventral view. **I**. Detail of the ad-cloacal pair of supplements. **J**. Caudal region in ventral view. Scale bars = A–C, E = 2 µm; D = 20 µm; F, I = 1 µm; G–H = 10 µm; J = 5 µm.

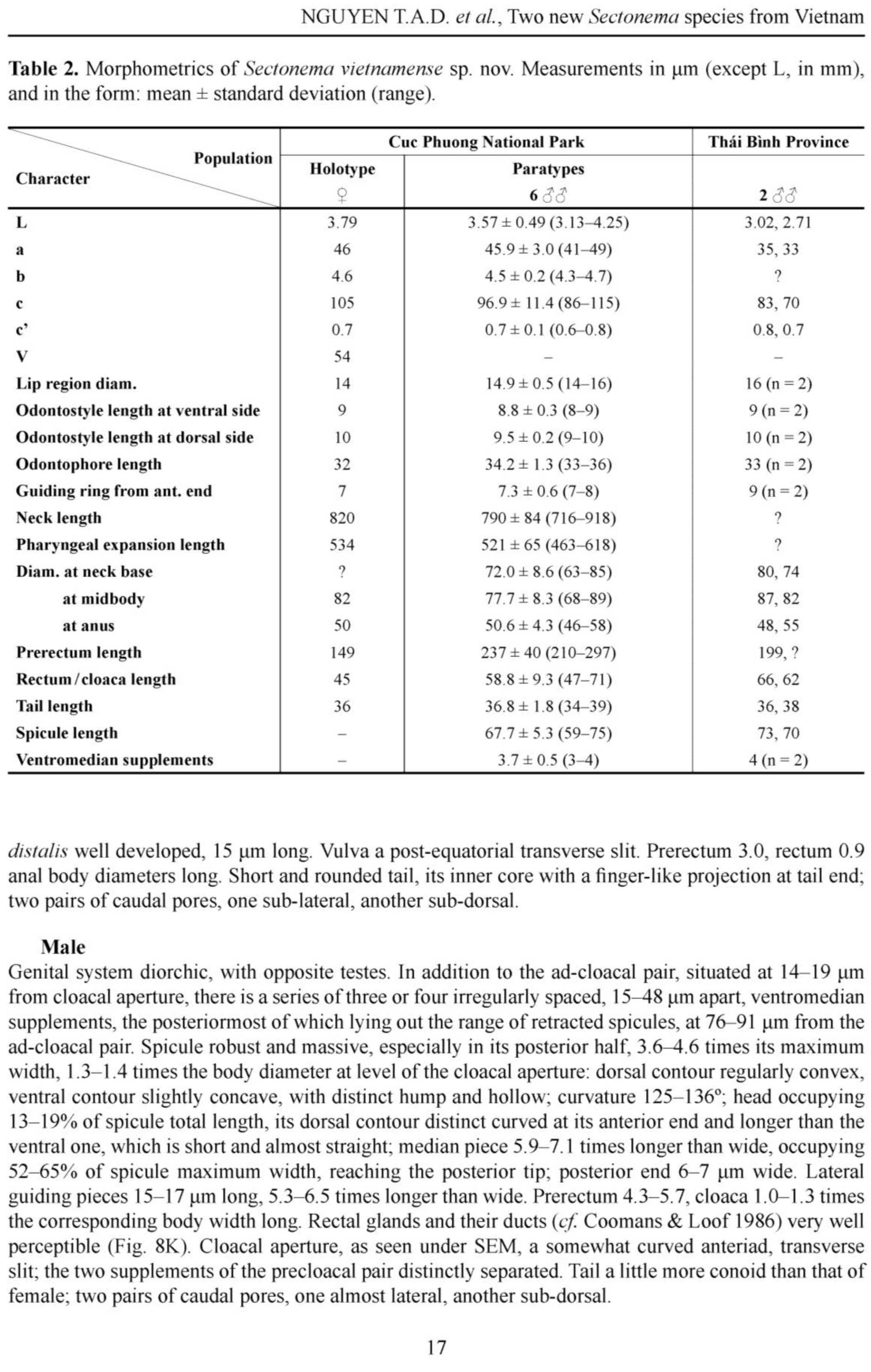

Table 2. Morphometrics of *Sectonema vietnamense* sp. nov. Measurements in µm (except L, in mm), and in the form: mean ± standard deviation (range).

Character / Population	Cuc Phuong National Park		Thái Bình Province
	Holotype	Paratypes	
	♀	6 ♂♂	2 ♂♂
L	3.79	3.57 ± 0.49 (3.13–4.25)	3.02, 2.71
a	46	45.9 ± 3.0 (41–49)	35, 33
b	4.6	4.5 ± 0.2 (4.3–4.7)	?
c	105	96.9 ± 11.4 (86–115)	83, 70
c’	0.7	0.7 ± 0.1 (0.6–0.8)	0.8, 0.7
V	54	–	–
Lip region diam.	14	14.9 ± 0.5 (14–16)	16 (n = 2)
Odontostyle length at ventral side	9	8.8 ± 0.3 (8–9)	9 (n = 2)
Odontostyle length at dorsal side	10	9.5 ± 0.2 (9–10)	10 (n = 2)
Odontophore length	32	34.2 ± 1.3 (33–36)	33 (n = 2)
Guiding ring from ant. end	7	7.3 ± 0.6 (7–8)	9 (n = 2)
Neck length	820	790 ± 84 (716–918)	?
Pharyngeal expansion length	534	521 ± 65 (463–618)	?
Diam. at neck base	?	72.0 ± 8.6 (63–85)	80, 74
at midbody	82	77.7 ± 8.3 (68–89)	87, 82
at anus	50	50.6 ± 4.3 (46–58)	48, 55
Prerectum length	149	237 ± 40 (210–297)	199, ?
Rectum / cloaca length	45	58.8 ± 9.3 (47–71)	66, 62
Tail length	36	36.8 ± 1.8 (34–39)	36, 38
Spicule length	–	67.7 ± 5.3 (59–75)	73, 70
Ventromedian supplements	–	3.7 ± 0.5 (3–4)	4 (n = 2)

distalis well developed, 15 µm long. Vulva a post-equatorial transverse slit. Prerectum 3.0, rectum 0.9 anal body diameters long. Short and rounded tail, its inner core with a finger-like projection at tail end; two pairs of caudal pores, one sub-lateral, another sub-dorsal.

Male

Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 14–19 µm from cloacal aperture, there is a series of three or four irregularly spaced, 15–48 µm apart, ventromedian supplements, the posteriormost of which lying out the range of retracted spicules, at 76–91 µm from the ad-cloacal pair. Spicule robust and massive, especially in its posterior half, 3.6–4.6 times its maximum width, 1.3–1.4 times the body diameter at level of the cloacal aperture: dorsal contour regularly convex, ventral contour slightly concave, with distinct hump and hollow; curvature 125–136º; head occupying 13–19% of spicule total length, its dorsal contour distinct curved at its anterior end and longer than the ventral one, which is short and almost straight; median piece 5.9–7.1 times longer than wide, occupying 52–65% of spicule maximum width, reaching the posterior tip; posterior end 6–7 µm wide. Lateral guiding pieces 15–17 µm long, 5.3–6.5 times longer than wide. Prerectum 4.3–5.7, cloaca 1.0–1.3 times the corresponding body width long. Rectal glands and their ducts (*cf.* Coomans & Loof 1986) very well perceptible (Fig. 8K). Cloacal aperture, as seen under SEM, a somewhat curved anteriad, transverse slit; the two supplements of the precloacal pair distinctly separated. Tail a little more conoid than that of female; two pairs of caudal pores, one almost lateral, another sub-dorsal.

Relationships

The new species is easily distinguishable from its congeners in the unique combination of these features: narrow and nearly continuous lip region, very short odontostyle, absence of *pars refringens vaginae* and short and rounded tail. Nonetheless, it resembles *S. barbatum* Heyns, 1965 in its general morphology, but it can be distinguished from this because of its oral field lacking any setae-like structure (vs bearing numerous short forwardly pointing setae), narrower (14–16 vs about 22 µm broad) lip region, shorter (8–9 vs 16 µm) odontostyle, *pars refringens vaginae* absent (vs present), shorter (36 µm, $c = 105$, $c' = 0.7$ vs 48 µm, $c = 81$, $c' = 1.0$) female tail, and male present (vs absent). In having a comparatively small body size and nearly continuous lip region, *S. vietnamense* sp. nov. is close to *S. mucrodens* and *S. truxum*, from which it differs in its narrower (14–16 vs more than 20 µm broad) lip region, shorter stomatal protruding structure (8–9 vs more than 20 µm) located as usual (vs the anterior tip distinctly behind the level of guiding ring), and *pars refringens vaginae* absent (vs consisting of two small pieces). *Sectonema vietnamense* sp. nov. is also similar to *S. pseudoventrale* Heyns, 1965 in having a short odontostyle, but can be distinguished from this by its narrower (14–16 vs 21–23 µm broad) lip region, shorter (209–242 µm or 2.5–2.9 times the corresponding body diameter vs 123 µm or 1.3 times the corresponding body diameter) uterus, shorter (36 µm, $c = 105$, $c' = 0.7$ vs 46–63 µm, $c = 82–130$, $c' = 0.8–1.0$) female tail, shorter (59–75 vs 90–95 µm) spicules, and fewer (three or four vs six or seven) ventromedian supplements. Finally, the new species also resembles *S. tropicum* sp. nov., but it can be distinguished from this by its narrower (14–16 vs 19–21 µm broad) lip region, shorter (8–9 vs 20–21 µm at its ventral side) odontostyle, longer (occupying 63–67 vs 52–59% of total neck length) pharyngeal expansion, more posterior vulva ($V = 54$ vs $V = 48–52$), *pars refringens vaginae* absent (vs present), shorter (59–75 vs 91–97 µm) spicules and higher number of ventromedian supplements (three or four vs one).

Remarks

The two males from Thái Bình are very similar to those of the type population, but some minor morphometric differences have been also noted: somewhat smaller (2.71, 3.02 vs 3.13–4.25 mm long) and more obese ($a = 33, 35$ vs $a = 41–49$) body. Such differences have been regarded as instraspecific variation as the number of specimens examined is low and the main morphological and morphometric features are coincident.

Acknowledgements

We would like to thank the directors of Cuc Phuong National Park and Cao Bang Nature Reserve for issuing relevant permits. This research was supported by the project entitled Aporcelaimidae Mundi: Revisión de la familia Aporcelaimidae Heyns, 1965 (Nematoda, Dorylaimida) (ref. CGL2012-33239; co-financed FEDER), the German Academic Exchange Service (DAAD) (PKZ 91540366), the Zoological Institute (University of Cologne) and IDEAL-WILD (United State). We thank Dr. Tam Vu for providing the specimens from Thai Binh and Idea-Wild (United States of America) for supporting the first author during her master thesis. SEM pictures were obtained with the assistance of technical staff and equipment of "Centro de Instrumentación Científico-Técnica (CICT)", University of Jaén.

References

Abolafia J. & Peña-Santiago R. 2005. Nematodes of the order Rhabditida from Andalucía Oriental: *Pseudacrobeles elongatus* (de Man, 1880) comb. n. *Nematology* 7 (6): 917–926. http://dx.doi.org/10.1163/156854105776186415

Álvarez-Ortega S., Nguyen T.A.D., Abolafia J., Vu T.T.T. & Peña-Santiago R. 2015. Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), and an updated taxonomy of the genus. *ZooKeys* 516: 1–26. http://dx.doi.org/10.3897/zookeys.516.10087

Álvarez-Ortega S. & Peña-Santiago R. 2013. Taxonomy of the genus *Aporcelaimellus* Heyns, 1965 (Nematoda, Dorylaimida, Aporcelaimidae). *Zootaxa* 3669: 243–260. http://dx.doi.org/10.11646/zootaxa.3669.3.3

Álvarez-Ortega S., Subbotin S.A. & Peña-Santiago R. 2013a. Morphological and molecular characterization of Californian species of the genus *Metaporcelaimus* Lordello, 1965 (Dorylaimida, Aporcelaimidae), with a new concept of the genus. *Nematology* 15 (3): 251–278. http://dx.doi.org/10.1163/15685411-00002674

Álvarez-Ortega S., Subbotin S.A. & Peña-Santiago R. 2013b. Morphological and molecular characterization of Californian species of the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae). *Nematology* 15 (4): 431–439. http://dx.doi.org/10.1163/15685411-00002691

Baermann G. 1917. Eine einfache Methode zur Auffindung von *Ankylostomum* (Nematoden) Larven in Erdproben. *Geneeskunding Tijdschrift voor Nederlandsch-Indië* 57: 131–137.

Coomans A. & Loof P.A.A. 1986. Observations on the glands of the male reproductive system in dorylaims and its phylogenetic importance. *Revue de Nématologie* 9 (3): 261–265.

Flegg J.J.M. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology* 60 (3): 429–437. http://dx.doi.org/10.1111/j.1744-7348.1967.tb04497.x

Holterman M., Rybarczyk K., van den Elsen S., van Megen H., Mooyman P., Peña-Santiago R., Bongers T., Bakker J. & Helder J. 2008. A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* 8 (1): 23–34. http://dx.doi.org/10.1111/j.1471-8286.2007.01963.x

Huelsenbeck J.P. & Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8): 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Loof P.A.A. & Coomans A. 1970. On the development and location of the oesophageal gland nuclei in the Dorylaimina. *Proceedings of the IX^th^ International Nematology Symposium (Warsaw, Poland, 1967)*: 79–161.

Nicholas K.B., Nicholas Jr H.B. & Deerfield II D.W. 1997. GeneDoc: analysis and visualization of genetic variation. *EMBnet News* 4: 1–14.

Nylander J.A.A. 2002. *MrModeltest v1.0b*. Department of Systematic Zoology, Uppsala University.

Peña-Santiago R., Abolafia J. & Álvarez-Ortega S. 2014. New proposal for a detailed description of the dorylaim spicule (Nematoda: Dorylaimida). *Nematology* 16 (9): 1091–1095. http://dx.doi.org/10.1163/15685411-00002834

Peña-Santiago R. & Álvarez-Ortega S. 2014a. Studies on the genus *Sectonema* Thorne, 1930 (Dorylaimida: Aporcelaimidae). Re-description of *S. ventrale*, the type species of the genus. *Nematology* 16 (9): 1097–1104. http://dx.doi.org/10.1163/15685411-00002837

Peña-Santiago R. & Álvarez-Ortega S. 2014b. Re-description of three species of the genus *Sectonema* Thorne, 1930 (Nematoda: Dorylaimida: Aporcelaimidae) originally studied by E. Altherr. *Zootaxa* 3881: 63–74. http://dx.doi.org/10.11646/zootaxa.3881.1.5

Ronquist F. & Huelsenbeck J. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12): 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Siddiqi M.R. 1964. Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 2: 174–184. http://dx.doi.org/10.1111/j.1439-0469.1964.tb00720.x

Subbotin S.A., Sturhan D., Chizhov V.N., Vovlas N. & Baldwin J.G. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8 (3): 455–474. http://dx.doi.org/10.1163/156854106778493420

Swofford D.L. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4.0b 10. Sunderland, MA, USA, Sinauer Associates.

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. http://dx.doi.org/10.1093/molbev/mst197

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. & Higgins D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25 (24): 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876

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Three new species of *Sectonema* Thorne, 1930 (Dorylaimida:Aporcelaimidae) from Vietnam

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Three new species of *Sectonema* Thorne, 1930 (Dorylaimida: Aporcelaimidae) from Vietnam

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Summary – Three new species of the genus *Sectonema* collected from natural habitats in Vietnam are studied, described and illustrated, including line drawings, LM and/or SEM pictures. *Sectonema birrucephalum* sp. n. is characterised by its 2.73-4.35 mm long body, lip region 18-20 μm broad and offset by deep constriction, odontostyle 10.0-11.5 μm long on its ventral side, 659-989 μm long neck, pharyngeal expansion occupying 63-68% of total neck length, uterus a simple tube, 221-277 μm long, *pars refringens vaginae* present, V = 54-56, tail short (31-43 μm, c = 85-111, c′ = 0.6-0.8) and rounded, spicules 72-75 μm long, and four or five irregularly spaced ventromedian supplements beyond the range of the spicules. *Sectonema buccociliatum* sp. n. is distinguished by its 2.00-2.46 mm long body, lip region offset by constriction, 19-20 μm broad and bearing perioral cilia-like structures, odontostyle 13-14 μm long at its ventral side, 530-625 μm long neck, pharyngeal expansion occupying 62-69% of total neck length, uterus a simple tube, 116-152 μm long, *pars refringens vaginae* present, V = 56-62, tail short (23-31 μm, c = 72-104, c′ = 0.6-0.8) and rounded, spicules 56-68 μm long, and 3-5 spaced and weakly developed ventromedian supplements beyond the range of the spicules. *Sectonema ciliatum* sp. n. is characterised by its 2.79-3.13 mm long body, lip region offset by constriction, 21-22 μm broad and bearing perioral cilia-like structures, odontostyle 14-15 μm long at its ventral side, 699-722 μm long neck, pharyngeal expansion occupying 60% of total neck length, uterus a simple tube, 201-244 μm long, *pars refringens vaginae* present, V = 52-53, tail short (33-35 μm, c = 82-92, c′ = 0.6-0.7) and rounded, spicules 70-72 μm long, and three or four spaced and weakly developed ventromedian supplements beyond the range of the spicules. Molecular data obtained for *S. ciliatum* sp. n. and the derived evolutionary tree show a close phylogenetic relationship with other species of the genus.

Keywords – D2-D3, description, LSU ribosomal DNA, molecular, morphology, morphometrics, phylogeny, *Sectonema birrucephalum* sp. n., *Sectonema buccociliatum* sp. n., *Sectonema ciliatum* sp. n., SEM, taxonomy.

This is the third in a series of contributions devoted to the study of species of Aporcelaimidae Heyns, 1965 from natural areas of Vietnam. The first contribution (Álvarez-Ortega *et al.*, 2015) dealt with three new species of *Aporcelaimoides* Heyns, 1965 whilst the second (Nguyen *et al.*, 2016) included two atypical species of *Sectonema* Thorne, 1930 characterised by having a nearly continuous lip region, an unusual feature in this taxon.

Nematological surveys conducted during the last 5 years yielded several specimens that fitted the general (typical) pattern of *Sectonema*, which, among other features, is currently defined on the basis of a lip region offset by a deep constriction and the stomatal protruding structure being either a reduced odontostyle or a mural tooth (see detailed discussion on this matter by Peña-Santiago & Álvarez-Ortega, 2014a). A detailed study of available material revealed it consisted of three unknown species of the genus, these being described herein.

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Materials and methods

NEMATODES

Nematodes were collected from natural areas in Vietnam, extracted from soil samples using the methods of Baermann (1917) and Flegg (1967), relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerin following Siddiqi's (1964) technique, and mounted on permanent glass slides to allow handling and observation under light microscopy (LM).

LIGHT MICROSCOPY

Measurements of specimens were taken using a light Olympus BH-2 microscope equipped with differential interference contrast (DIC). Morphometrics included de Man's indices and most of the usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof & Coomans (1970) and spicule terminology follows Peña-Santiago *et al.* (2014). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope equipped with DIC and a Nikon DS digital camera. Images were edited using Adobe® Photoshop® CS. Drawings were made using a *camera lucida*.

SCANNING ELECTRON MICROSCOPY

After their examination and identification, a few specimens preserved in glycerin were selected for observation under SEM following the protocol of Abolafia & Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point-dried, coated with gold, and observed with a Zeiss Merlin microscope.

DNA EXTRACTION, PCR AND SEQUENCING

Fresh material for molecular analyses was first available for only one of the three species and no new specimens were collected in later field surveys. DNA was extracted from single individuals using the proteinase K protocol. A nematode specimen was transferred to a 0.5 ml Eppendorf tube containing 18 μl of Worm Lysis Buffer (WLB) (50 mM KCL, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40, and 0.45% Tween 20) and 2 μl proteinase K (600 μg ml^{-1}) (Thermo Scientific). The tubes were incubated at 65°C (1 h) and then at 95°C (15 min). PCR and sequence protocols were as described in detail by Nguyen *et al.* (2016). The primers used for amplification of D2-D3 region of 28S rRNA gene were D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.*, 2006). The sequences obtained were submitted to the GenBank database under accession numbers KU589226 and KU589227.

PHYLOGENETIC ANALYSES

The newly obtained sequences were aligned with other 46 D2-D3 expansion segments of 28S rRNA gene sequences available in GenBank using ClustalX 1.83 (Thompson *et al.*, 1997). Outgroup taxa were chosen according to the results of previous published data (Holterman *et al.*, 2008; Álvarez-Ortega *et al.*, 2013a). Sequence alignments were manually edited using GenDoc 2.6.002 (Nicholas *et al.*, 1997). The sequence dataset was analysed with Bayesian inference (BI) and Maximum Likelihood (ML) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and MEGA 6 (Tamura *et al.*, 2013), respectively. The best fit model of DNA evolution for BI was obtained using the program MrModeltest 2.3 (Nylander, 2004) with the Akaike Information Criterion in conjunction with PAUP* 4b10 (Swofford, 2003). BI analysis under the GTR + G + I model was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 10^6 generations. ML analysis was implemented under the best-fitting evolutionary model (GTR + I + G), obtained using the program MEGA 6, and 1000 bootstrap replications. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. The trees were visualised with the program FigTree v1.4.0 and drawn with Adobe Acrobat XI Pro 11.0.1.

Results

Sectonema birrucephalum[*] **sp. n.**

(Figs 1-3)

MATERIAL EXAMINED

Six females and five males, from two locations, in acceptable state of preservation.

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[*] The specific epithet is a composite derived from the Latin root *birrus* = beret, and the Greek root *kephalé* = head, and refers to the lip region morphology.

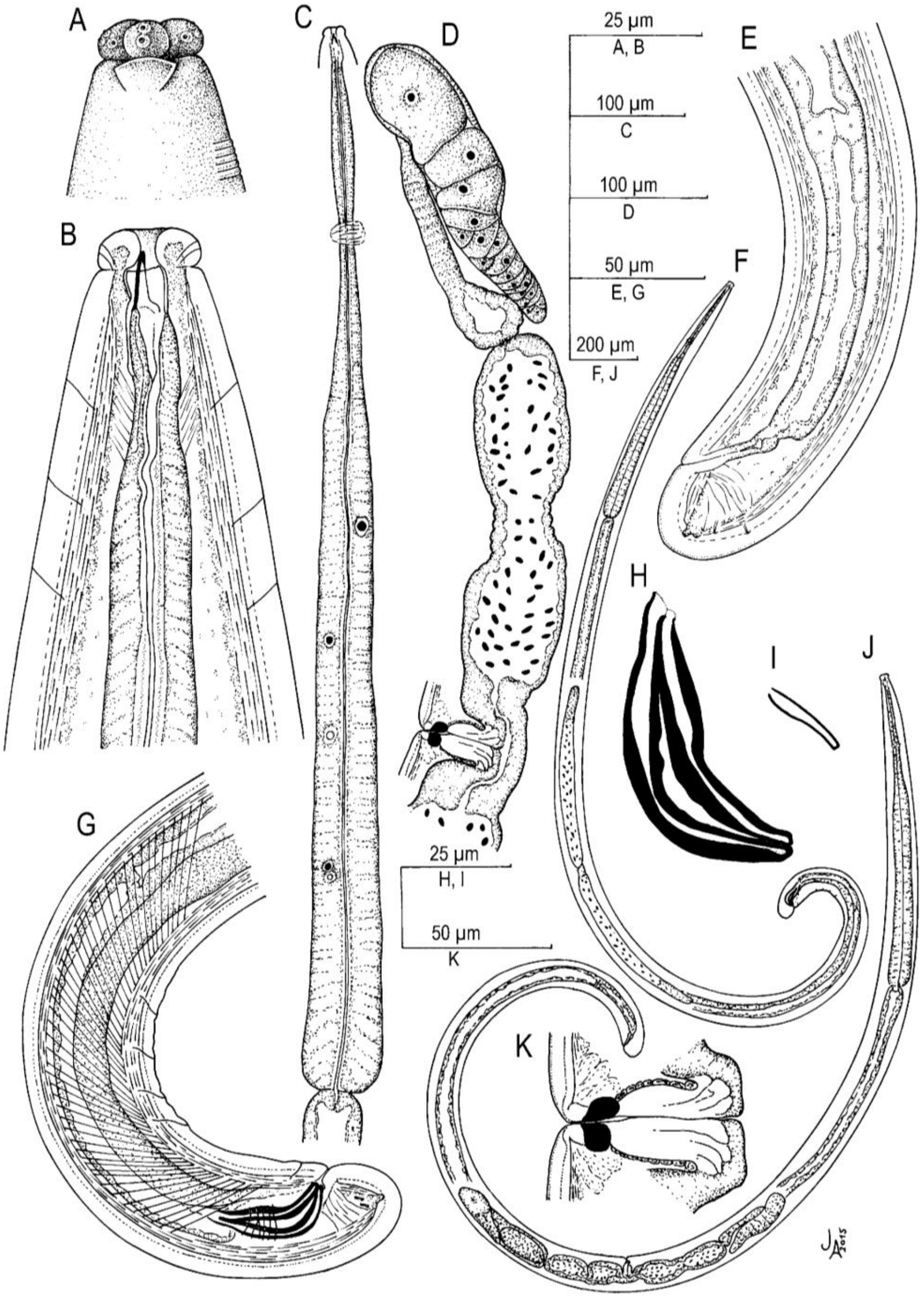


Fig. 1. *Sectonema birrucephalum* sp. n. A: Lip region in lateral surface view; B: Anterior region in lateral median view; C: Pharynx; D: Female, anterior genital branch; E: Female, posterior body region; F: Male, entire; G: Male, posterior body region; H: Spicule; I: Lateral guiding piece; J: Female, entire; K: Vagina.

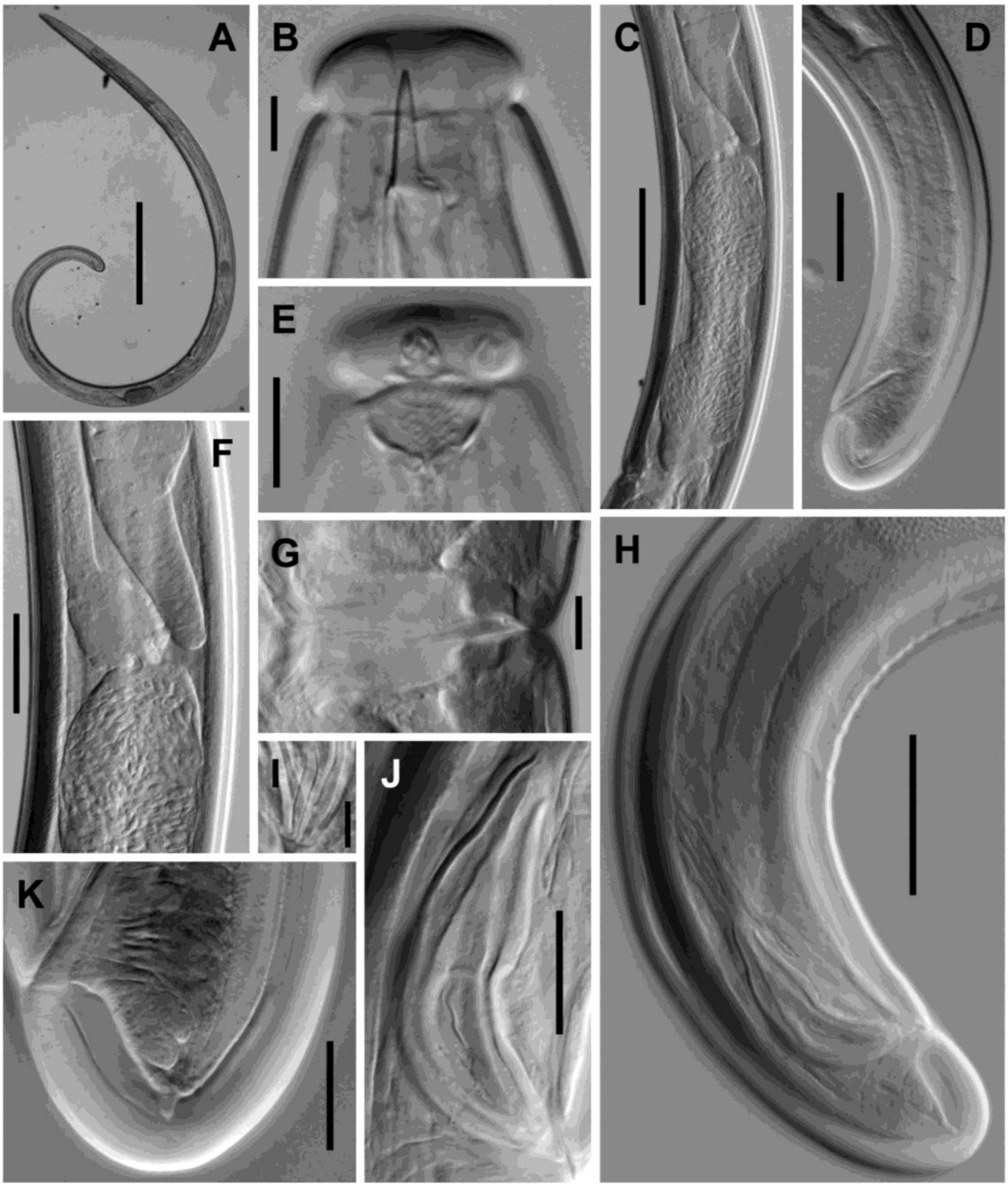


Fig. 2. *Sectonema birrucephalum* sp. n. (LM, type population). A: Female, entire; B: Anterior region in median view; C: Female, anterior genital branch; D: Female, posterior body region; E: Lip region in lateral surface view; F: Female, oviduct-uterus junction; G: Vagina; H: Male, posterior body region; I: Lateral guiding piece; J: Spicule; K: Female, caudal region. (Scale bars: A = 500 μm; B = 5 μm; C = 100 μm; D, E, G, I = 10 μm; F, H = 50 μm; J, K = 20 μm.)

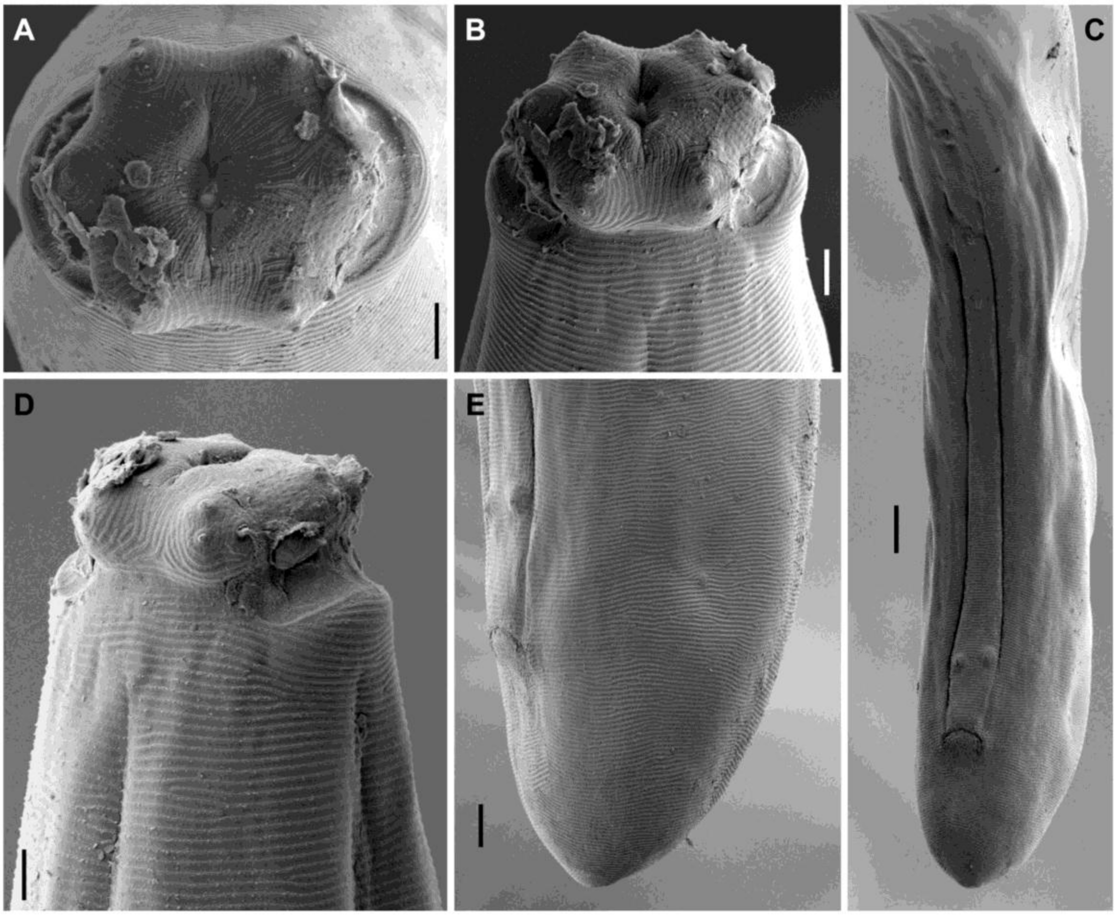


Fig. 3. *Sectonema birrucephalum* sp. n. (SEM, male from Thai Binh province). A: Lip region in frontal view; B: Lip region in ventral view; C: Posterior body region in ventral view; D: Lip region in sublateral view; E: Caudal region in sublateral view. (Scale bars: A, B, D = 2.5 μm; C = 10 μm; E = 5 μm.)

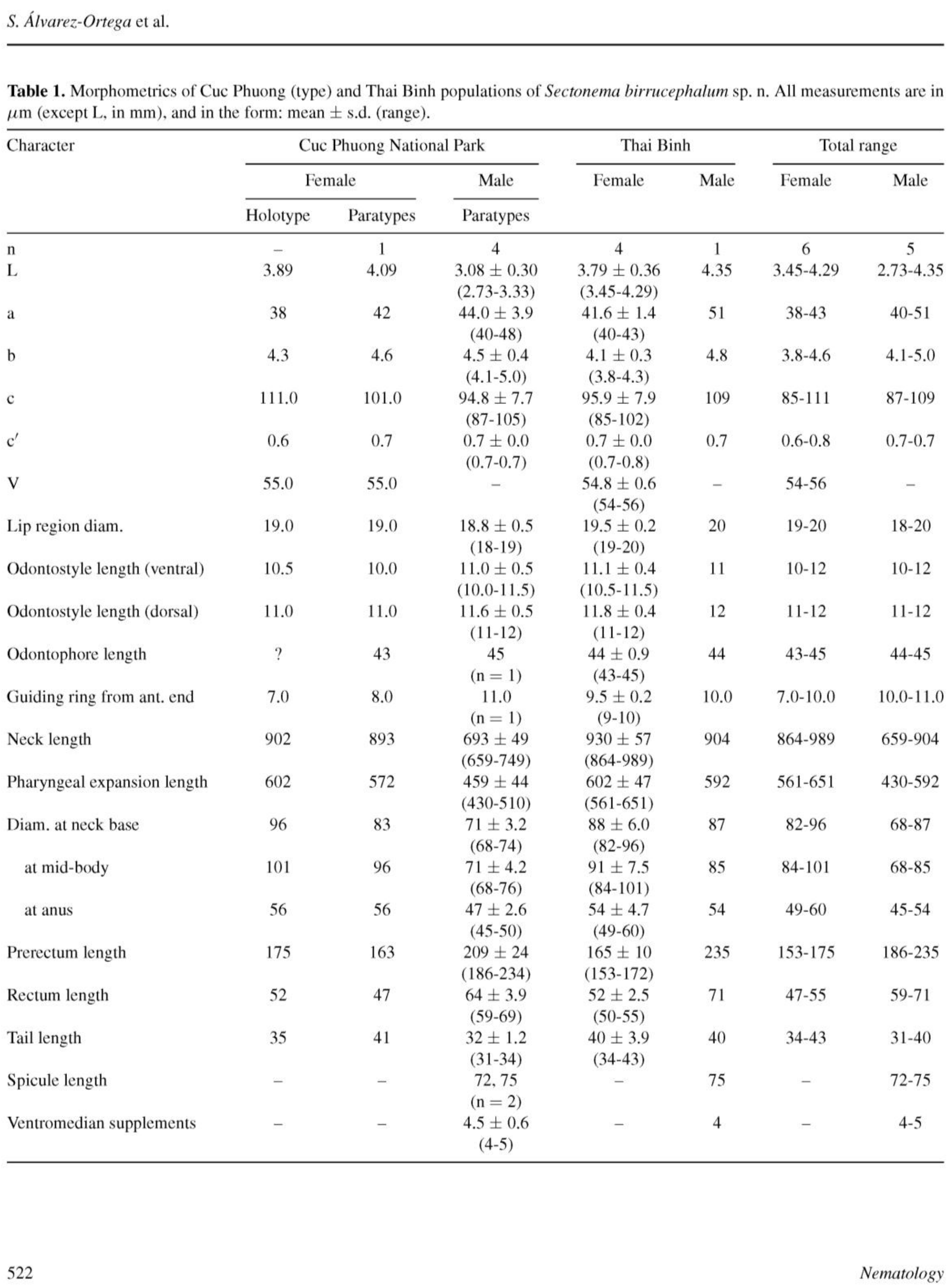
MEASUREMENTS

See Table 1.

DESCRIPTION

Adults

Slender to very slender nematodes of medium to large size, 2.73-4.35 mm long. Body cylindrical, distinctly tapering towards anterior end, less so towards posterior because caudal region is rounded. Habitus regularly curved ventrad after fixation, to a more or less open C, occasionally more curved in posterior body region, and spiral-shaped in single male specimen. Cuticle three-layered, especially visible at caudal region, consisting of thinner outer layer bearing very fine transverse striations over entire body, thicker intermediate layer with radial striation and thin inner layer, thickness 3-4 μm in anterior region, 4.0-5.5 μm in mid-body and 7-10 μm on tail. Lateral chord 7-16 μm wide at mid-body, occupying one-tenth to one-sixth (9-16%) of mid-body diam. Body pores comparatively large, two ventral and two dorsal pores often present at level of stoma, their corresponding ducts appearing especially thickened beneath inner and intermedi-



S. *Álvarez-Ortega* et al.

Table 1. Morphometrics of Cuc Phuong (type) and Thai Binh populations of *Sectonema birrucephalum* sp. n. All measurements are in μm (except L, in mm), and in the form: mean ± s.d. (range).

Character	Cuc Phuong National Park			Thai Binh		Total range	
	Female		Male	Female	Male	Female	Male
	Holotype	Paratypes	Paratypes				
n	–	1	4	4	1	6	5
L	3.89	4.09	3.08 ± 0.30 (2.73-3.33)	3.79 ± 0.36 (3.45-4.29)	4.35	3.45-4.29	2.73-4.35
a	38	42	44.0 ± 3.9 (40-48)	41.6 ± 1.4 (40-43)	51	38-43	40-51
b	4.3	4.6	4.5 ± 0.4 (4.1-5.0)	4.1 ± 0.3 (3.8-4.3)	4.8	3.8-4.6	4.1-5.0
c	111.0	101.0	94.8 ± 7.7 (87-105)	95.9 ± 7.9 (85-102)	109	85-111	87-109
c′	0.6	0.7	0.7 ± 0.0 (0.7-0.7)	0.7 ± 0.0 (0.7-0.8)	0.7	0.6-0.8	0.7-0.7
V	55.0	55.0	–	54.8 ± 0.6 (54-56)	–	54-56	–
Lip region diam.	19.0	19.0	18.8 ± 0.5 (18-19)	19.5 ± 0.2 (19-20)	20	19-20	18-20
Odontostyle length (ventral)	10.5	10.0	11.0 ± 0.5 (10.0-11.5)	11.1 ± 0.4 (10.5-11.5)	11	10-12	10-12
Odontostyle length (dorsal)	11.0	11.0	11.6 ± 0.5 (11-12)	11.8 ± 0.4 (11-12)	12	11-12	11-12
Odontophore length	?	43	45 (n = 1)	44 ± 0.9 (43-45)	44	43-45	44-45
Guiding ring from ant. end	7.0	8.0	11.0 (n = 1)	9.5 ± 0.2 (9-10)	10.0	7.0-10.0	10.0-11.0
Neck length	902	893	693 ± 49 (659-749)	930 ± 57 (864-989)	904	864-989	659-904
Pharyngeal expansion length	602	572	459 ± 44 (430-510)	602 ± 47 (561-651)	592	561-651	430-592
Diam. at neck base	96	83	71 ± 3.2 (68-74)	88 ± 6.0 (82-96)	87	82-96	68-87
at mid-body	101	96	71 ± 4.2 (68-76)	91 ± 7.5 (84-101)	85	84-101	68-85
at anus	56	56	47 ± 2.6 (45-50)	54 ± 4.7 (49-60)	54	49-60	45-54
Prerectum length	175	163	209 ± 24 (186-234)	165 ± 10 (153-172)	235	153-175	186-235
Rectum length	52	47	64 ± 3.9 (59-69)	52 ± 2.5 (50-55)	71	47-55	59-71
Tail length	35	41	32 ± 1.2 (31-34)	40 ± 3.9 (34-43)	40	34-43	31-40
Spicule length	–	–	72, 75 (n = 2)	–	75	–	72-75
Ventromedian supplements	–	–	4.5 ± 0.6 (4-5)	–	4	–	4-5

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ate cuticle layers. Lip region rounded or somewhat cap-like, offset by deep constriction, 2.7-3.0 times as wide as high and one-fifth to two-sevenths (19-28%) of body diam. at neck base, apparently (under SEM) marked by radial (oral field) or longitudinal (remaining lip region) incisures, with amalgamated lips and protruding, pore-like labial and cephalic papillae, all surrounded by a ring-like annulus, oral field perceptibly hexagonal, oral aperture a dorso-ventral slit, lip region therefore showing bi-radial symmetry. Amphid fovea cup-shaped, its opening occupying 11.5-12.5 μm or three-fifths to two-thirds (61-66%) of lip region diam. Cheilostom nearly cylindrical, lacking any differentiation. Stomatal protruding structure in form of a reduced odontostyle 0.5-0.6 times as long as lip region diam. on its ventral side and 0.24-0.42% of body length. Guiding ring simple, somewhat plicate, located at 0.4-0.5 lip region diam. from anterior end. Odontophore linear, rod-like, 3.7-4.2 times odontostyle length. Anterior region of pharynx enlarging very gradually, basal expansion 9.1-11.7 times as long as broad, 6.2-7.4 times as long as body diam., occupying 63-68% of total neck length. Pharyngeal gland nuclei located as follows: DO = 41-43 (n = 4); DN = 45-48 (n = 5); S_1N_1 = 57, 58 (n = 2); S_1N_2 = 66-67 (n = 3); S_2N = 78-80 (n = 6). Nerve ring located at 144-207 μm from anterior end or 20-22% of total neck length. Cardia rounded conoid, nearly as long as wide, 12-18 × 12-19 μm, a weak ring-like structure present surrounding its junction with pharyngeal base.

Female

Genital system didelphic-amphidelphic, with both branches almost equally and well developed, anterior 420-491 μm long or 11-13% of body length (484, 509 μm (n = 2) long or 11, 12% of body length with one uterine egg inside), and posterior 407-537 μm long or 11-14% of body length (574 μm (n = 1) long or 14% of body length with two uterine eggs inside). Ovaries variably sized, usually reaching sphincter level, anterior 106-245 μm, posterior 107-230 μm long, oocytes arranged first in two or more rows, then in a single row. Oviduct 120-150 μm long or 1.3-1.7 times corresponding body diam. (273 μm (n = 1) long or 2.7 times corresponding body diam. with one egg inside), and consisting of a slender part made of prismatic cells and a well developed *pars dilatata*. Oviduct-uterus junction marked by a sphincter. Uterus a simple tube-like structure 221-277 μm long or 2.2-3.3 times corresponding body diam. (248, 282 μm long (n = 2) or 2.6, 2.8 times corresponding body diam. with one egg, and 340 μm (n = 1) long or 3.5 times corresponding body diam. with two uterine eggs), usually containing abundant sperm cells inside. Uterine eggs ovoid, 121-133 × 69-75 μm, 1.6-1.9 times as long as wide. Vagina extending inwards 43-49 μm or about half (45-50%) of body diam., *pars proximalis* 28-34 × 24-28 μm, with somewhat sigmoid walls surrounded by weak musculature, *pars refringens* with two drop-shaped pieces measuring 9-11 × 6-8 μm and with a combined width of 14-18 μm, *pars distalis* 4-6 μm long. Vulva a post-equatorial transverse slit. Prerectum 2.8-3.2 and rectum 0.8-1.0 anal body diam. long. Tail short and rounded, inner core bearing a finger-like projection at tail end. Two pairs of caudal pores, one lateral, another subdorsal.

Male

Genital system diorchic, with opposed testes. In addition to adcloacal pair, situated at 15-19 μm from cloacal aperture, a series of four or five irregularly spaced, 13-38 μm apart, ventromedian supplements, posteriormost of which lying out of range of spicules at 63-95 μm from adcloacal pair. Spicules robust (n = 3), 3.9-4.0 times their max. width long, 1.4-1.5 times body diam. at level of cloacal aperture, dorsal contour regularly convex, ventral contour concave, with shallow hump and hollow, curvature 132-135°, head occupying 9-11% of spicule total length, its dorsal side longer than ventral, median piece 5.6-6.4 times as long as wide, occupying 57-63% of spicule max. width, reaching posterior tip, posterior end 7 μm wide. Lateral guiding pieces 20 μm (n = 3) long, 6.3-7.1 times as long as wide. Prerectum 4.1-4.7 and cloaca 1.3-1.4 times corresponding body diam. long. Rectal glands and their ducts (*cf.*, Coomans & Loof, 1986) very clear. Cloacal aperture (under SEM) a curved anteriad, transverse slit. Tail similar to that of female. Two pairs of caudal pores, one lateral, one subdorsal.

TYPE HABITAT AND LOCALITY

Soil from a pristine tropical forest with *Dracontomelum* sp. and *Tetrameles* sp. as dominant plant species, Cuc Phuong National Park, Ninh Binh Province, Northern Vietnam (GPS coordinates: 20°19′00″N, 105°36′40″E).

OTHER HABITAT AND LOCALITY

Rhizosphere of *Zea mays* L., Thai Binh Province, Northern Vietnam.

TYPE MATERIAL

Female holotype and three male paratypes deposited in the nematode collection of the University of Jaén, Spain. One female and one male paratype deposited in the nematode collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam.

DIAGNOSIS AND RELATIONSHIPS

The new species is characterised by its 2.73-4.35 mm long and slender (a = 38-51) body, lip region offset by deep constriction and 18-20 μm broad, odontostyle 10.0-11.5 μm long on its ventral side, neck 659-989 μm long, pharyngeal expansion 430-651 μm long or occupying 63-68% of total neck length, uterus a simple tube-like structure 221-277 μm long or 2.2-3.3 times the corresponding body diam., *pars refringens vaginae* present, V = 54-56, female tail short (34-43 μm, c = 85-111, c′ = 0.6-0.8) and rounded, male tail similar to that of female (31-40 μm, c = 87-109, c′ = 0.7), spicules 72-75 μm long, and four or five irregularly and well-spaced ventromedian supplements lying outside the spicule range.

In having a short, reduced odontostyle (10.0-11.5 μm on its ventral side) and short tail (c′ = 0.6-0.8) the new species is morphologically close to *S. brevicauda* Heyns, 1965, *S. macrospiculum* (Altherr, 1958) Heyns, 1965, *S. pseudoventrale* Heyns, 1965 and *S. ventrale* Thorne, 1930, but can be distinguished from all of them by its narrower lip region (18-20 *vs* >20 μm). In addition, *S. birrucephalum* sp. n. differs from *S. brevicauda* by its smaller general size (L = 2.73-4.35 *vs* 7.73 mm, neck 659-989 *vs* 1333 μm long), more posterior vulva (V = 54-56 *vs* 51), longer female tail (31-40 μm, c = 87-109, c′ = 0.7 *vs* 28 μm, c = 280, c′ = 0.4), and male present *vs* unknown. From *S. macrospiculum* (see recent redescription by Peña-Santiago & Álvarez-Ortega, 2014b) by its more slender body (a = 38-51 *vs* 35-37), shorter odontostyle (10.0-11.5 *vs* 15 μm on its ventral side), comparatively longer uterus (2.2-3.3 *vs* 1.9-2.1 times corresponding body diam.), shorter (31-43 *vs* 58-62 μm) and rounded *vs* convex conoid to conoid tail, shorter spicules (72-75 *vs* 107 μm), smaller lateral guiding pieces (20 *vs* 25 μm), and fewer ventromedian supplements (four or five *vs* nine). From *S. pseudoventrale* by its longer odontostyle (10.0-11.5 *vs ca* 8.5 μm on its ventral side), longer uterus (221-277 *vs* 123 μm, 2.2-3.3 *vs* 1.3 times corresponding body diam.), comparatively shorter female tail (c′ = 0.6-0.8 *vs* 0.8-1.0), shorter spicules (72-75 *vs* 90-95 μm), smaller lateral guiding pieces (20 *vs* 28 μm), and fewer ventromedian supplements (four or five *vs* six or seven); and from *S. ventrale* (see recent redescription by Peña-Santiago & Álvarez-Ortega, 2014a) by its smaller general size (L = 2.73-4.35 *vs* 7.09-10.42 mm, neck 659-989 *vs* 1180-1474 μm), shorter odontostyle (10.0-10.5 *vs* 12-14 μm on its ventral side), pharyngeal expansion (63-68 *vs* 68-73% of neck length), uterus (221-277 *vs* 363-623 μm), female tail (31-40 *vs* 47-71 μm), spicules (72-75 *vs* 120 μm) and lateral guiding pieces (20 *vs* 33 μm), and fewer ventromedian supplements (four or five *vs* eight or nine). Moreover, the new species also resembles *S. vietnamense* Nguyen, Abolafia, Bonkowski, Peña-Santiago & Álvarez-Ortega, 2016, but differs from it in the wider lip region (18-20 *vs* 14-16 μm) offset by deep constriction *vs* depression, and *pars refringens vaginae* well developed *vs* absent.

REMARKS

Although the specimens examined were collected from two different locations they are very similar morphologically and morphometrically and no remarkable difference was found between them.

***Sectonema buccociliatum*[*] sp. n.**
(Figs 4-6)

MATERIAL EXAMINED

Five females and five males from one location, in acceptable state of preservation.

MEASUREMENTS

See Table 2.

DESCRIPTION

Adults

Slender to very slender nematodes of medium size, 2.00-2.46 mm long. Body cylindrical, distinctly tapering towards anterior end, less so towards posterior as caudal region is rounded. Habitus regularly curved ventrad after fixation, C- or G-shaped, occasionally more curved in posterior body region, spiral-shaped in only one female specimen. Cuticle three-layered, especially distinguishable in caudal region, consisting of thinner outer layer bearing very fine transverse striation through entire body, thicker intermediate layer with radial striation and

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[*] The specific epithet is a compound term referring to the presence of cilia-like structures in the anterior part of the stoma.

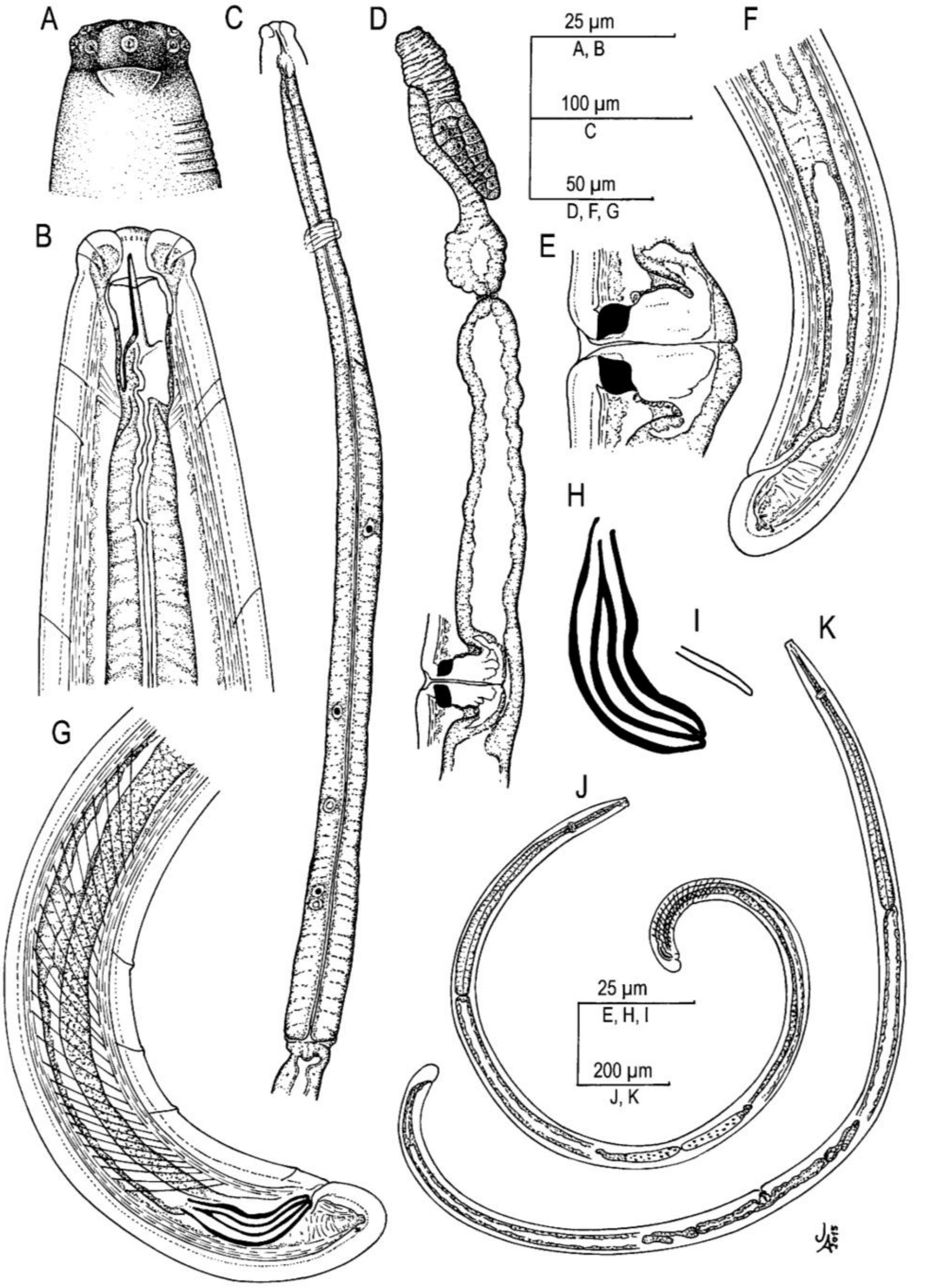

Fig. 4. *Sectonema buccociliatum* sp. n. A: Lip region in lateral surface view; B: Anterior region in lateral median view; C: Pharynx; D: Female, anterior genital branch; E: Vagina; F: Female, posterior body region; G: Male, posterior body region; H: Spicule; I: Lateral guiding piece; J: Male, entire; K: Female, entire.

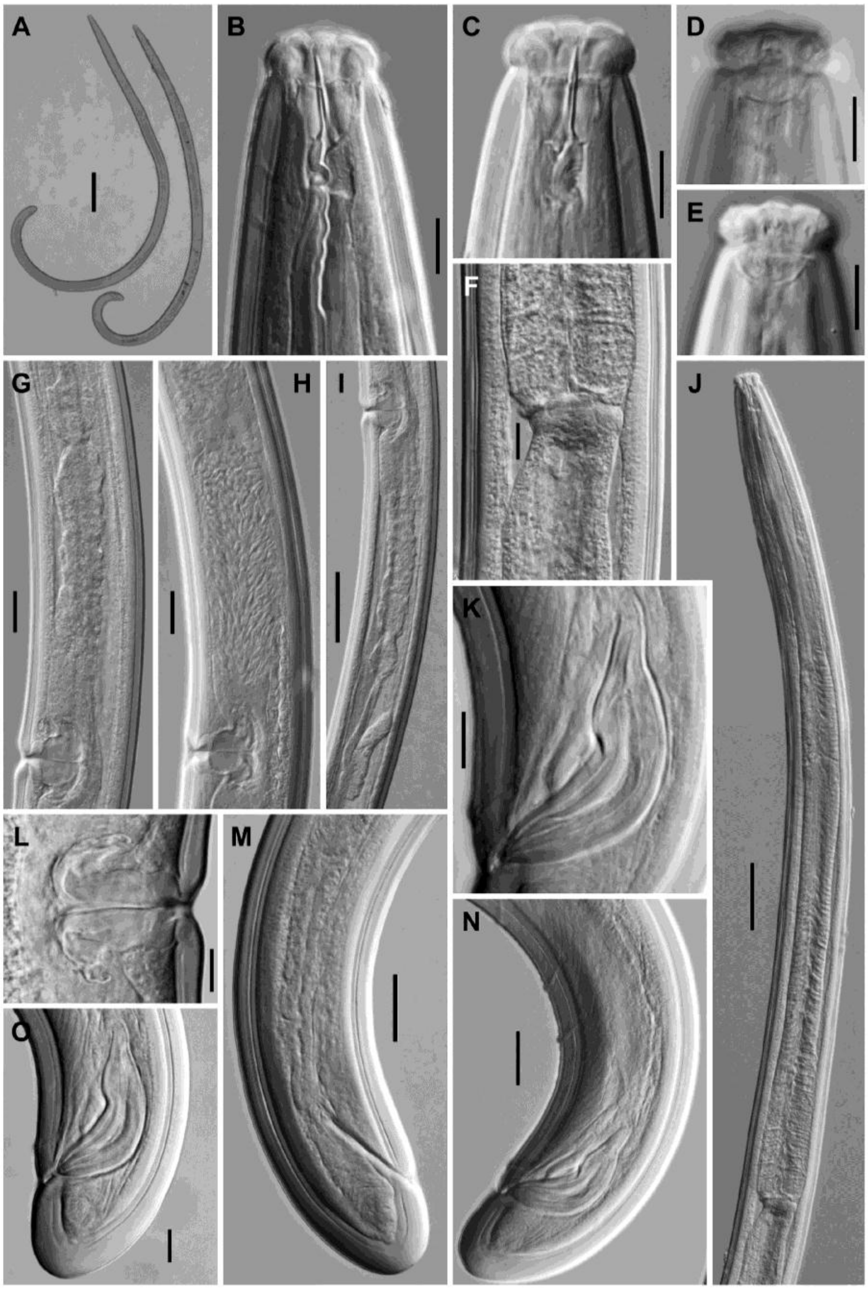

Fig. 5. *Sectonema buccociliatum* sp. n. (LM). A: Female and male, entire; B, C: Anterior region in lateral median view; D, E: Lip region in lateral surface view; F: Pharyngo-intestinal junction; G, H: Anterior uterus; I: Female, posterior genital branch; J: Neck region; K: Spicule; L: Vagina; M: Female, posterior body region; N: Male, posterior body region; O: Male, caudal region and spicule. (Scale bars: A = 200 μm; B-F, K, L, O = 10 μm; G, H, M, N = 20 μm; I, J = 50 μm.)

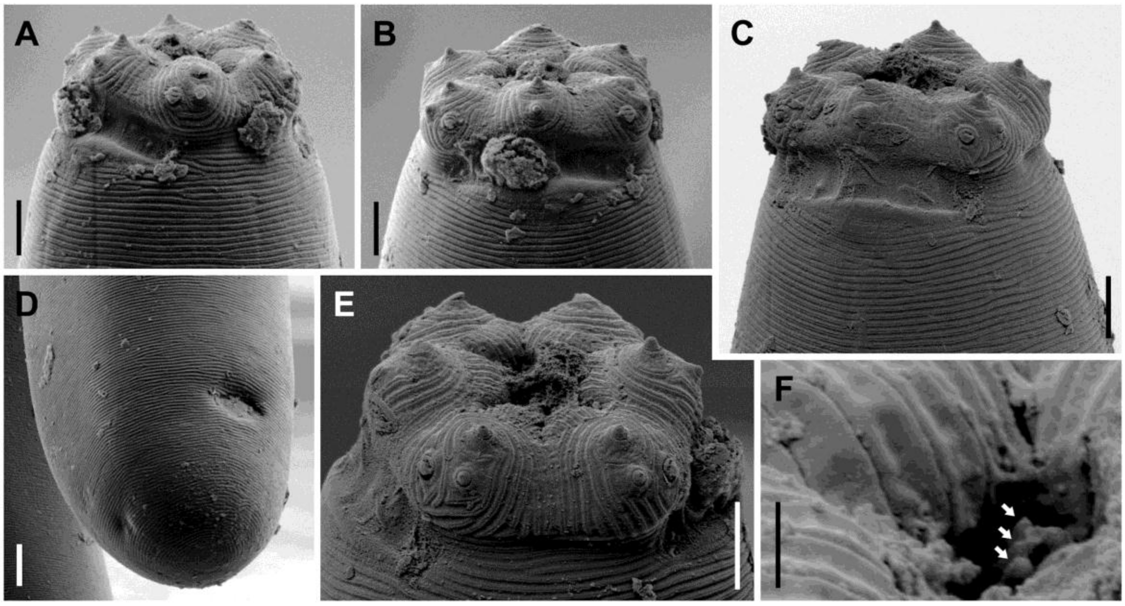

Fig. 6. *Sectonema buccociliatum* sp. n. (SEM). A, C: Lip region in sublateral view; B: Lip region in lateral view; D: Female, caudal region in sublateral view; E: Lip region in ventral view; F: Detail of oral aperture, showing cilia-like structures at anterior end of cheilostom (arrows). (Scale bars: A-C, E = 3 μm; D = 5 μm; F = 0.5 μm.)

thin inner layer, thickness 4-5 μm in anterior region, 4-6 μm in mid-body and 8-10 μm on tail. Lateral chord 6-13 μm wide at mid-body, occupying one-tenth to one-fifth (10-21%) of mid-body diam. Two ventral and two dorsal body pores usually present at level of odontophore, their corresponding ducts appearing especially thickened beneath intermediate cuticle layer. Lip region offset by constriction, 2.6-2.9 times as wide as high and one-fourth to less than two-fifths (27-37%) of body diam. at neck base, lips (under SEM) mostly amalgamated, but their perioral part distinctly separated by existence of six radial, interlabial, marked incisures, button-like and protruding labial papillae, all of them surrounded by a ring-like annulus, cephalic papillae pore- rather than button-like, showing a short transverse slit-like opening, and also surrounded by a ring-like annulus, oral field visibly hexagonal, with dorso-ventral aperture showing presence of short cilia-like structures at anterior part of cheilostom. Amphid fovea cup-shaped, its opening occupying 11-12 μm or *ca* three-fifths (56-63%) of lip region diam. Cheilostom nearly cylindrical, with cilia-like structures at its anterior part. Odontostyle typical of genus, its ventral side 0.7 times as long as lip region diam. or 0.54-0.68% of body length. Guiding ring simple, somewhat plicate, at 0.4-0.5 lip region diam. from anterior end. Odontophore linear, rod-like, 1.8 (n = 1) times odontostyle length. Anterior region of pharynx enlarging very gradually, basal expansion 7.5-10.9 times as long as broad, 5.7-7.2 times as long as body diam., occupying 62-69% of total neck length. Pharyngeal gland nuclei located as follows (n = 1): DO = 46, DN = 50, S_1N_1 = 68, S_1N_2 = 77, S_2N = 86. Nerve ring located at 127-139 μm from anterior end or 21-25% of total neck length. Cardia rounded conoid, 13-15 × 10-14 μm, prominent ring-like structure surrounding junction to pharyngeal base.

Female

Genital system didelphic-amphidelphic, with both branches almost equally and well developed, anterior 221-281 μm long or 10-12% of body length and posterior 219-269 μm long or 9-11% of body length. Ovaries variably sized, anterior 69-123 μm, posterior 60-119 μm long, oocytes arranged first in two or more rows, then in a single row. Oviduct 71-95 μm long or 1.1-1.6 times corresponding body diam., consisting of a slender part made of prismatic cells and a well-developed *pars dilatata* with

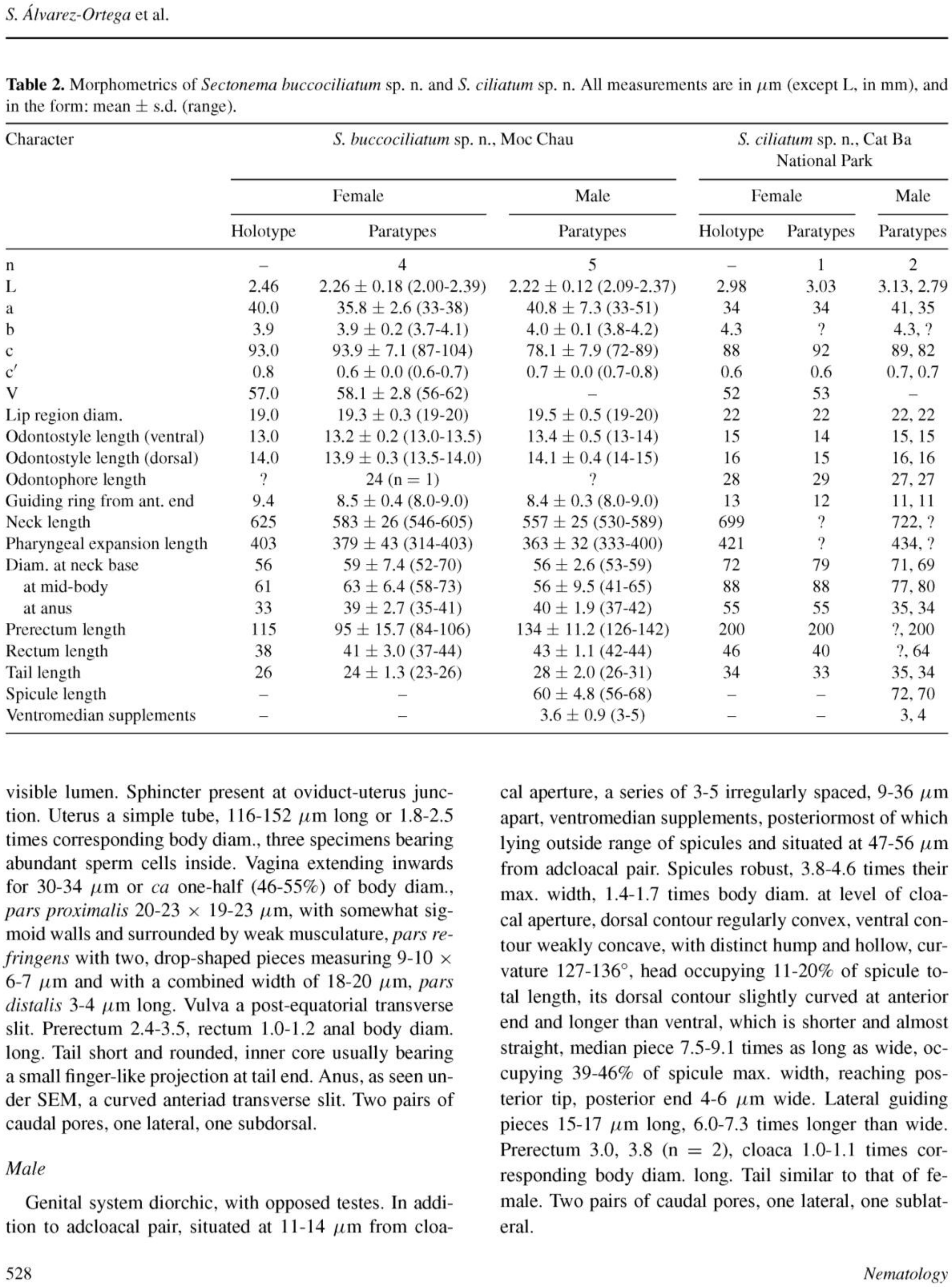

Table 2. Morphometrics of *Sectonema buccociliatum* sp. n. and *S. ciliatum* sp. n. All measurements are in μm (except L, in mm), and in the form: mean ± s.d. (range).

Character	*S. buccociliatum* sp. n., Moc Chau			*S. ciliatum* sp. n., Cat Ba National Park		
	Female		Male	Female		Male
	Holotype	Paratypes	Paratypes	Holotype	Paratypes	Paratypes
n	–	4	5	–	1	2
L	2.46	2.26 ± 0.18 (2.00-2.39)	2.22 ± 0.12 (2.09-2.37)	2.98	3.03	3.13, 2.79
a	40.0	35.8 ± 2.6 (33-38)	40.8 ± 7.3 (33-51)	34	34	41, 35
b	3.9	3.9 ± 0.2 (3.7-4.1)	4.0 ± 0.1 (3.8-4.2)	4.3	?	4.3, ?
c	93.0	93.9 ± 7.1 (87-104)	78.1 ± 7.9 (72-89)	88	92	89, 82
c′	0.8	0.6 ± 0.0 (0.6-0.7)	0.7 ± 0.0 (0.7-0.8)	0.6	0.6	0.7, 0.7
V	57.0	58.1 ± 2.8 (56-62)	–	52	53	–
Lip region diam.	19.0	19.3 ± 0.3 (19-20)	19.5 ± 0.5 (19-20)	22	22	22, 22
Odontostyle length (ventral)	13.0	13.2 ± 0.2 (13.0-13.5)	13.4 ± 0.5 (13-14)	15	14	15, 15
Odontostyle length (dorsal)	14.0	13.9 ± 0.3 (13.5-14.0)	14.1 ± 0.4 (14-15)	16	15	16, 16
Odontophore length	?	24 (n = 1)	?	28	29	27, 27
Guiding ring from ant. end	9.4	8.5 ± 0.4 (8.0-9.0)	8.4 ± 0.3 (8.0-9.0)	13	12	11, 11
Neck length	625	583 ± 26 (546-605)	557 ± 25 (530-589)	699	?	722, ?
Pharyngeal expansion length	403	379 ± 43 (314-403)	363 ± 32 (333-400)	421	?	434, ?
Diam. at neck base	56	59 ± 7.4 (52-70)	56 ± 2.6 (53-59)	72	79	71, 69
at mid-body	61	63 ± 6.4 (58-73)	56 ± 9.5 (41-65)	88	88	77, 80
at anus	33	39 ± 2.7 (35-41)	40 ± 1.9 (37-42)	55	55	35, 34
Prerectum length	115	95 ± 15.7 (84-106)	134 ± 11.2 (126-142)	200	200	?, 200
Rectum length	38	41 ± 3.0 (37-44)	43 ± 1.1 (42-44)	46	40	?, 64
Tail length	26	24 ± 1.3 (23-26)	28 ± 2.0 (26-31)	34	33	35, 34
Spicule length	–	–	60 ± 4.8 (56-68)	–	–	72, 70
Ventromedian supplements	–	–	3.6 ± 0.9 (3-5)	–	–	3, 4

visible lumen. Sphincter present at oviduct-uterus junction. Uterus a simple tube, 116-152 μm long or 1.8-2.5 times corresponding body diam., three specimens bearing abundant sperm cells inside. Vagina extending inwards for 30-34 μm or *ca* one-half (46-55%) of body diam., *pars proximalis* 20-23 × 19-23 μm, with somewhat sigmoid walls and surrounded by weak musculature, *pars refringens* with two, drop-shaped pieces measuring 9-10 × 6-7 μm and with a combined width of 18-20 μm, *pars distalis* 3-4 μm long. Vulva a post-equatorial transverse slit. Prerectum 2.4-3.5, rectum 1.0-1.2 anal body diam. long. Tail short and rounded, inner core usually bearing a small finger-like projection at tail end. Anus, as seen under SEM, a curved anteriad transverse slit. Two pairs of caudal pores, one lateral, one subdorsal.

Male

Genital system diorchic, with opposed testes. In addition to adcloacal pair, situated at 11-14 μm from cloacal aperture, a series of 3-5 irregularly spaced, 9-36 μm apart, ventromedian supplements, posteriormost of which lying outside range of spicules and situated at 47-56 μm from adcloacal pair. Spicules robust, 3.8-4.6 times their max. width, 1.4-1.7 times body diam. at level of cloacal aperture, dorsal contour regularly convex, ventral contour weakly concave, with distinct hump and hollow, curvature 127-136°, head occupying 11-20% of spicule total length, its dorsal contour slightly curved at anterior end and longer than ventral, which is shorter and almost straight, median piece 7.5-9.1 times as long as wide, occupying 39-46% of spicule max. width, reaching posterior tip, posterior end 4-6 μm wide. Lateral guiding pieces 15-17 μm long, 6.0-7.3 times longer than wide. Prerectum 3.0, 3.8 (n = 2), cloaca 1.0-1.1 times corresponding body diam. long. Tail similar to that of female. Two pairs of caudal pores, one lateral, one sublateral.

TYPE HABITAT AND LOCALITY

Soil from a natural forest in the Moc Chau highlands, Son La Province, Northern Vietnam.

TYPE MATERIAL

Female holotype, two female and four male paratypes deposited in the nematode collection of the University of Jaén, Spain. One male and one female paratype deposited in the nematode collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam.

DIAGNOSIS AND RELATIONSHIPS

The new species is characterised by its 2.00-2.46 mm long body, lip region 19-20 μm broad and offset by a constriction, cheilostom bearing cilia-like structures in its anterior part, odontostyle 13-14 μm long on its ventral side, 530-625 μm long neck, pharyngeal enlargement 314-403 μm long and occupying 62-69% of total neck length, uterus a simple tube-like structure 116-152 μm long or 1.8-2.5 times the body diam., *pars refringens vaginae* present, V = 56-62, short (23-26 μm, c = 87-104, c′ = 0.6-0.8) and rounded female tail, male tail similar to that of female (26-31 μm, c = 72-89, c′ = 0.7-0.8), spicules 56-68 μm long, and 3-5 spaced ventromedian supplements beyond the range of the spicules.

The new species can be distinguished from its congeners by its small size, the smallest within the genus. In having a small body (L = 2.00-2.46), short odontostyle (13-14 μm on its ventral side) and short tail (c′ = 0.6-0.8), *S. buccociliatum* sp. n. is morphologically close to *S. macrospiculum*, *S. pseudoventrale* and *S. vietnamense*, from which it differs in its smaller general size (body 2.00-2.46 *vs* >2.7 mm long) and the presence of cilia-like structures in the anterior cheilostom. In addition, it can be distinguished from *S. macrospiculum* by its narrower lip region (19-20 *vs* 27-28 μm), shorter neck (530-625 *vs* 823-935 μm), shorter uterus (116-152 *vs* 270-297 μm), more posterior vulva (V = 56-62 *vs* 53), shorter tail (23-31 *vs* 58-62 μm), lateral guiding pieces (15-17 *vs* 25 μm) and spicules (56-68 *vs* 107 μm), and fewer ventromedian supplements (3-5 *vs* eight). From *S. pseudoventrale* by its narrower lip region (19-20 *vs* 21-23 μm), longer odontostyle (13-14 *vs* 8.5 μm on its ventral side), shorter neck (530-625 *vs* 850-1072 μm), more posterior vulva (V = 56-62 *vs* 50-56), shorter lateral guiding pieces (15-17 *vs* 28 μm), tail (23-31 *vs* 37-63 μm) and spicules (56-68 *vs* 90-95), and fewer ventromedian supplements (3-5 *vs* six or seven); and from *S. vietnamense* by its lip region offset by a constriction *vs* depression and wider (19-20 *vs* 14-16 μm), longer odontostyle (13-14 *vs* 8-9 μm on its ventral side), shorter uterus (116-152 *vs* 209-242 μm or 1.8-2.5 *vs* 2.5-2.9 times body diam.), *pars refringens vaginae* present *vs* absent, more posterior vulva (V = 56-62 *vs* 54), and shorter tail (23-31 *vs* 34-39 μm).

Sectonema buccociliatum sp. n. also resembles *S. anisonchum* Siddiqi, 1984 and *S. basilgoodeyi* Heyns, 1965, but can be separated from both by its smaller size (L = 2.00-2.46 *vs* >3.9 mm), anterior part of cheilostom bearing *vs* lacking cilia-like structures, and the nature of the stomatal protruding structure (a reduced odontostyle *vs* a mural tooth). Moreover, the new species differs from *S. anisonchum* by its longer odontostyle (13-14 *vs* 10.0-10.5 μm on its ventral side), shorter neck (530-625 *vs* 960-1040 μm), female tail (23-26 *vs* 34-42 μm) and spicules (56-68 *vs* 84-96 μm), longer lateral guiding piece (15-17 *vs* 13-14 μm), and fewer ventromedian supplements (3-5 *vs* seven or eight); and from *S. basilgoodeyi* by its narrower lip region (19-20 *vs ca* 23 μm), shorter neck (530-625 *vs* 874-924 μm), shorter uterus (116-152 μm long or 1.8-2.5 times the body diam. *vs ca* 366 μm long or 3.4 times the body diam.), tail (23-31 *vs* 55 μm) and spicules (56-68 *vs* 110 μm), and fewer ventromedian supplements (3-5 *vs* eight).

Sectonema ciliatum [*] **sp. n.**

(Figs 7-10)

MATERIAL EXAMINED

Two females and two males from one location, in acceptable state of preservation.

MEASUREMENTS

See Table 2.

DESCRIPTION

Adults

Slender to very slender nematodes of medium to large size, 2.79-3.13 mm long. Body cylindrical, distinctly tapering towards anterior end, less so towards posterior as caudal region is short and rounded. Habitus curved

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[*] The specific epithet refers to the presence of cilia-like structures in the anterior part of the cheilostom.

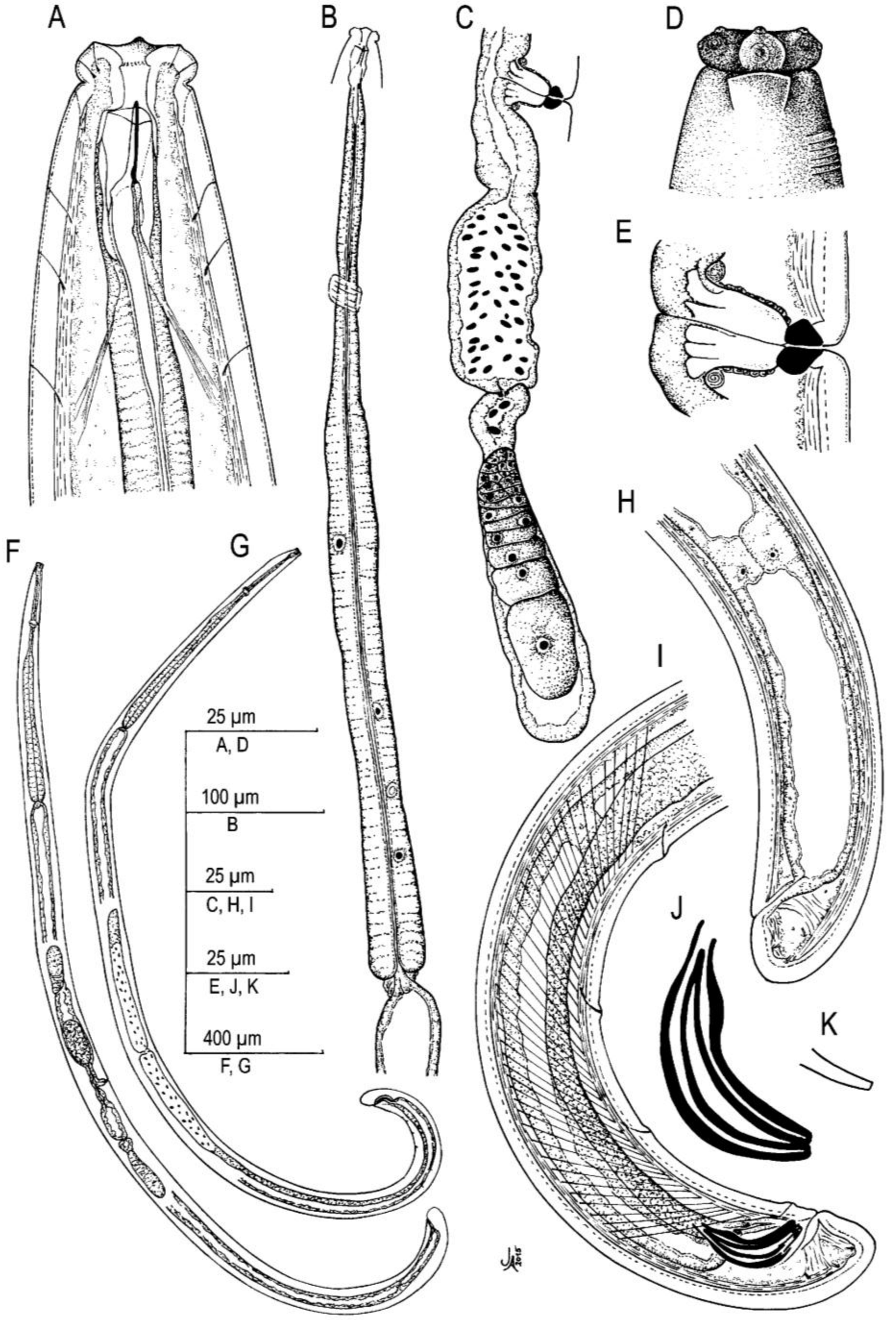

Fig. 7. *Sectonema ciliatum* sp. n. A: Anterior region lateral median view; B: Pharynx; C: Female, posterior genital branch; D: Lip region in lateral surface view; E: Vagina; F: Female, entire; G: Male, entire; H: Female, posterior body region; I: Male, posterior body region; J: Spicule; K: Lateral guiding piece.

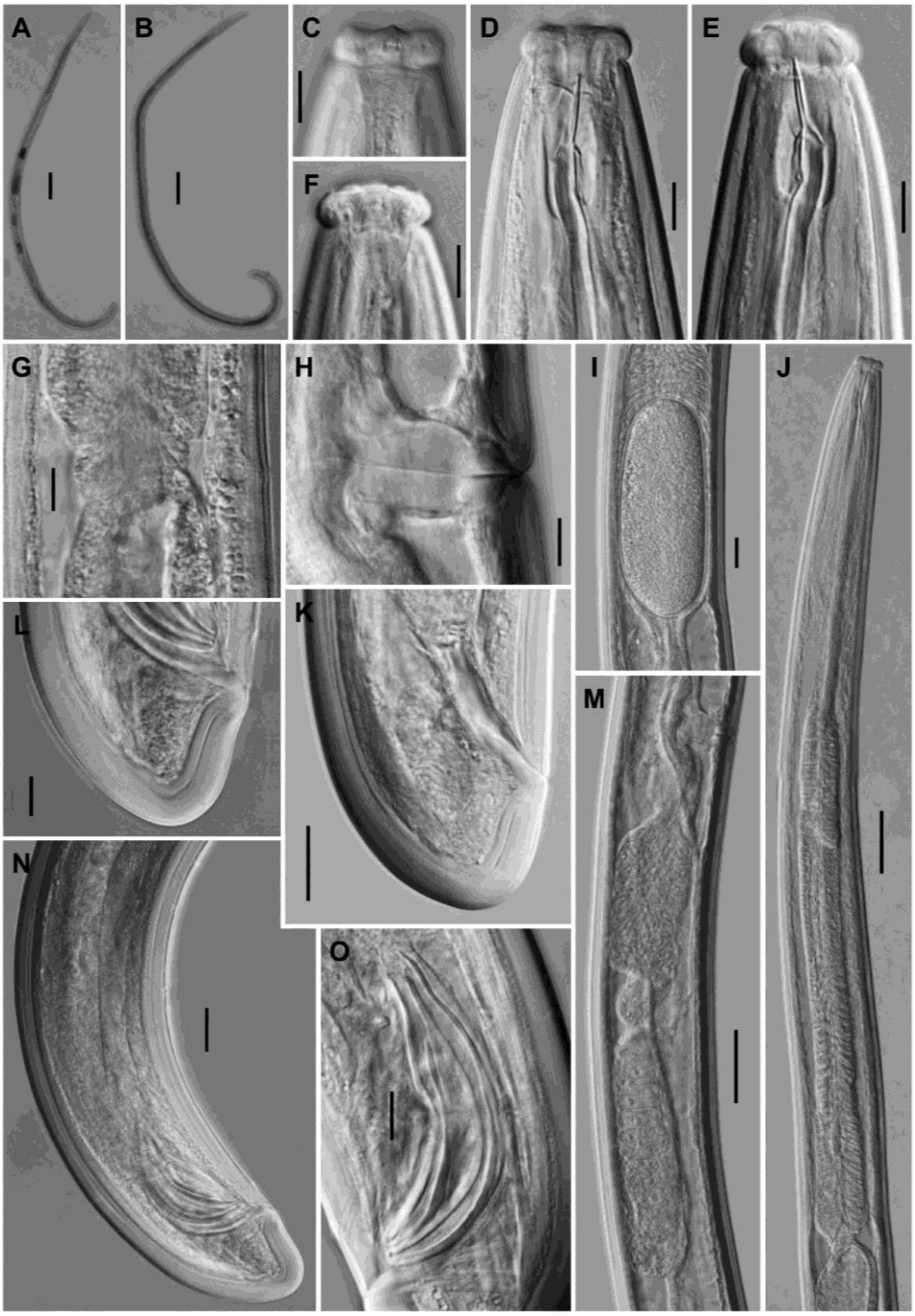

Fig. 8. *Sectonema ciliatum* sp. n. (LM). A: Female, entire; B: Male, entire; C, F: Lip region in lateral surface view; D, E: Lip region in lateral median view; G: Pharyngo-intestinal junction; H: Vagina; I: Uterine egg; J: Neck region; K: Female, caudal region; L: Male, caudal region; M: Female, posterior genital branch; N: Male, posterior body region; O: Spicule. (Scale bars: A, B = 200 μm; C-H, L, O = 10 μm; I, K, N = 20 μm; J, M = 50 μm.)

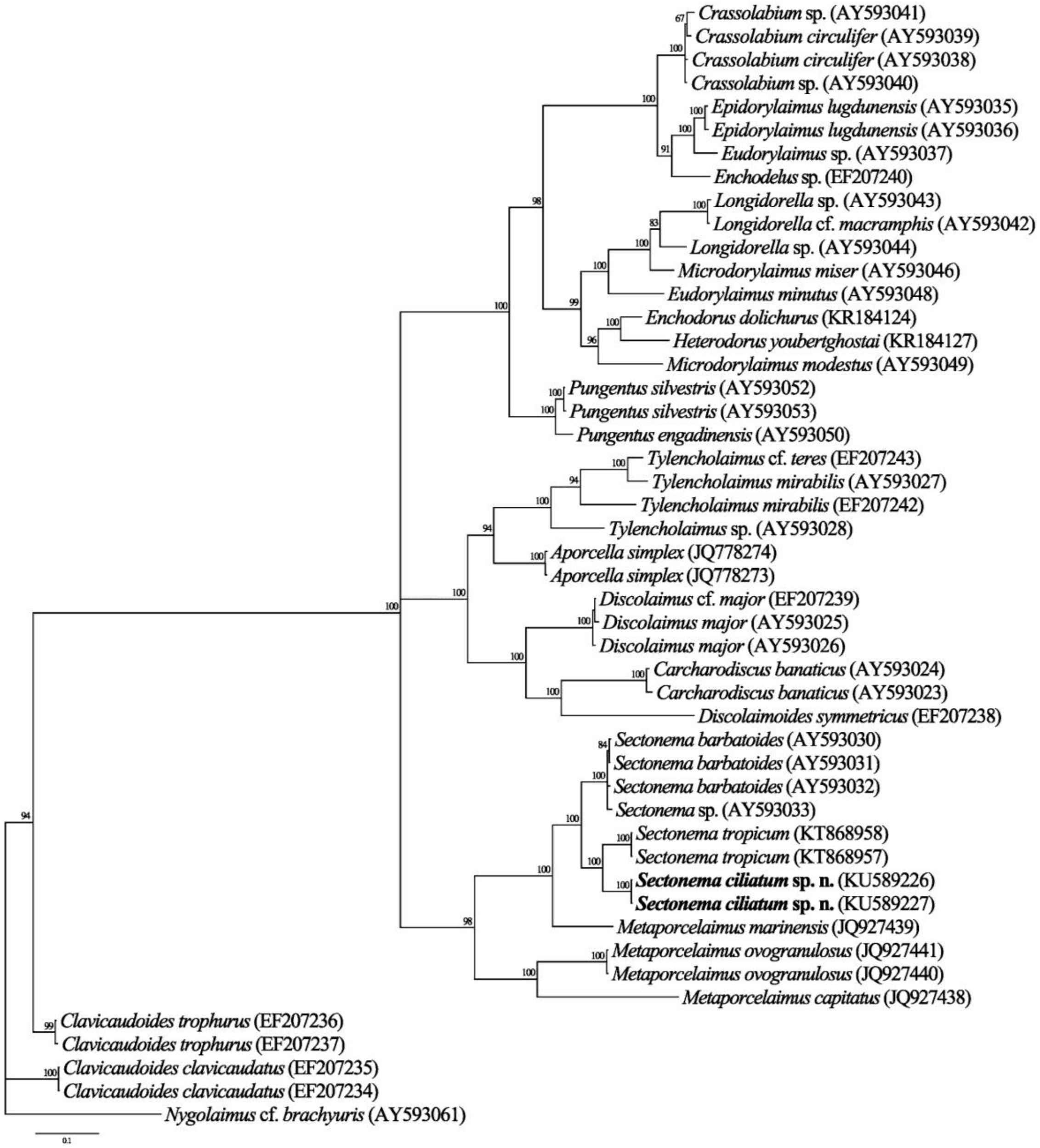

Fig. 9. Bayesian 50% majority rule consensus trees as inferred from D2-D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Posterior probabilities are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

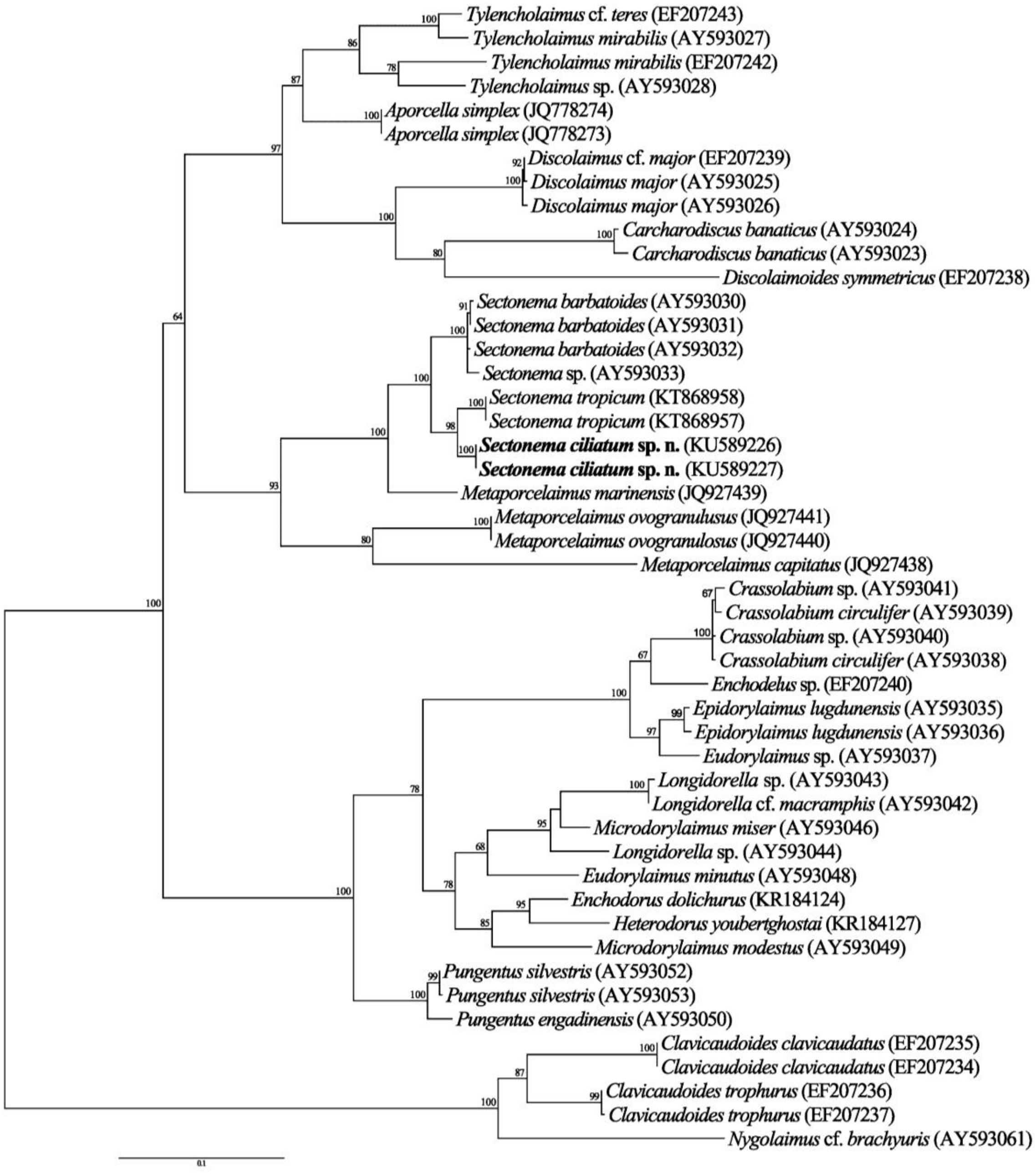

Fig. 10. Maximum Likelihood tree as inferred from D2-D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Bootstrap values are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

ventrad after fixation, especially in posterior body region, J-shaped in female, G-shaped in male. Cuticle three-layered, especially distinguishable in caudal region, consisting of thinner outer layer bearing very fine transverse striation throughout entire body, thicker intermediate layer with radial striation and thin inner layer, thickness 3.5-4.5 μm in anterior region, 4.5-5.0 μm at mid-body and 8-9 μm on tail. Lateral chord 15-17 μm wide at mid-body, occupying *ca* one-fifth (18-21%) of mid-body diam. Three ventral and three dorsal body pores often present at level of odontophore. Lip region offset by deep constriction, 2.9-3.2 times as wide as high and one-fourth to less than one-third (27-32%) of body diam. at neck base, lips mostly amalgamated, rounded, papillae distinct, somewhat protruding. Amphid fovea cup-shaped, its opening occupying 12.0-12.5 μm or five-ninths (55-57%) of lip region diam. Cheilostom nearly cylindrical, bearing cilia-like structures in its anterior part. Odontostyle typical of genus, its ventral side 0.7 times as long as lip region diam. or 0.47-0.52% of body length. Guiding ring simple, plicate, at 0.5-0.6 lip region diam. from anterior end. Odontophore linear, rod-like, 1.8-2.0 times odontostyle length. Anterior region of pharynx enlarging very gradually; basal expansion 10, 12 (n = 2) times longer than wide, 5.8, 6.1 (n = 2) times longer than body diam. and occupying 60% (n = 2) of total neck length. Pharyngeal gland nuclei obscure in most specimens examined and their location only observed in one specimen: DO = 49, DN = 54, $S_1N_1 = 72$, $S_1N_2 = 81$, $S_2N = 88$. Nerve ring located at 169-191 μm from anterior end or 24, 26% (n = 2) of total neck length. Cardia rounded conoid, 23 × 12 μm (n = 1), a conspicuous, somewhat asymmetrical ring-like structure present surrounding junction with pharyngeal base.

Female

Genital system didelphic-amphidelphic, with both branches almost equally and well developed, anterior not measured (not totally extended in one specimen and broken in another), posterior 375, 443 μm (n = 2) or 13, 15% of body length. Ovaries moderately sized, usually reaching sphincter level, anterior 182 μm (n = 1), posterior 169, 238 μm long, oocytes arranged first in two or more rows, then in a single row. Oviduct 126, 138 μm long or 1.4, 1.6 times corresponding body diam., consisting of a slender part made of prismatic cells and a well-developed *pars dilatata*. Oviduct-uterus junction marked by a sphincter. Uterus apparently a simple tube-like structure, 201 or 211 μm long, or 2.3 or 2.4 times corresponding body diam. (244 μm long or 2.8 times corresponding body diam., with one uterine egg inside), uterine eggs ovoid, 144-148 × 54-60 μm, 2.5-2.7 times as long as wide. Vagina extending inwards for 40 μm or less than one-half (45, 46%) of body diam., *pars proximalis* 27, 30 × 20 μm, with somewhat sigmoid walls surrounded by weak musculature, *pars refringens* with two, drop-shaped to trapezoidal pieces measuring 9, 10 × 7 μm and with a combined width of 14 or 15 μm, *pars distalis* 2.5 or 3 μm long. Vulva a post-equatorial transverse slit. Prerectum 3.7, rectum 0.7-0.8 anal body diam. long. Tail short and rounded. Two pairs of caudal pores, both nearly sublateral.

Male

Genital system diorchic, with opposed testes. In addition to adcloacal pair, situated at 14, 15 μm from cloacal aperture, a series of three or four widely spaced, 32-38 μm apart, and weakly developed ventromedian supplements, posteriormost of which lying beyond range of spicules and 99, 100 μm from adcloacal pair. Spicule robust, 4.6, 4.7 times its max. width, 1.4 (n = 2) times body diam. at level of cloacal aperture, dorsal contour regularly convex, ventral contour slightly concave, with shallow hump and hollow, curvature 127, 136°, head occupying 9, 11% of spicule total length, its dorsal contour hardly curved at anterior end and longer than ventral one, which is shorter and almost straight, median piece 8.0, 8.3 times as long as wide, occupying 51, 53% of spicule max. width, reaching posterior tip, posterior end 7 (n = 2) μm wide. Lateral guiding pieces 19, 20 μm long, 6.9, 7.2 times as long as wide. Prerectum 4.0 (n = 1), cloaca 1.3 (n = 1) times corresponding body diam. long. Tail similar to that of female. Two pairs of caudal pores, one lateral, one subdorsal.

TYPE HABITAT AND LOCALITY

Soil from a secondary forest with *Cinnamomum* sp. as dominant plant species, Cat Ba National Park, Hai Phong Province, Northern Vietnam (GPS coordinates: 20°47′45″N, 107°00′40″E; 170 m a.s.l.).

TYPE MATERIAL

Female holotype and one female and two male paratypes deposited in the nematode collection of the University of Jaén, Spain.

DIAGNOSIS AND RELATIONSHIPS

This new species is characterised by its 2.79-3.13 mm long body, lip region 21.5-22.0 μm broad and offset by constriction, anterior part of cheilostom bearing cilia-like structures, odontostyle 14-15 μm long on its ventral side, neck 699-722 μm long, pharyngeal enlargement 421-434 μm long or occupying 60% of total neck length, uterus apparently a simple tube-like structure 201-244 μm long or 2.3-2.8 times the body diam., *pars refringens vaginae* well developed, V = 52-53, short (33-35 μm, c = 82-92, c′ = 0.6-0.7) and rounded tail, spicules 70-72 μm long, and three or four widely spaced ventromedian supplements beyond the range of the spicules.

The new species is very similar to *S. buccociliatum* sp. n., but differs by its larger general size (L = 2.79-3.13 *vs* 2.00-2.46 mm, neck = 699-722 *vs* 530-625 μm), wider lip region (21-22 *vs* 19-20 μm), more anterior vulva (V = 52-53 *vs* 56-62), and inner core lacking *vs* usually bearing a finger-like projection at tail end.

In having a small body, short odontostyle (14-15 μm on its ventral side) and short tail (c′ up to 1.0), *S. ciliatum* sp. n. is close to *S. macrospiculum*, *S. pseudoventrale* and *S. vietnamense*, but differs from all by the presence *vs* absence of cilia-like structures in the anterior cheilostom. In addition, it can be distinguished from *S. macrospiculum* by its smaller general size (L = 2.79-3.13 *vs* 4.07-5.06 mm, neck = 699-722 *vs* 823-935 μm), narrower lip region (21-22 *vs* 27-28 μm), shorter pharyngeal expansion (60 *vs* 67-68% of total neck length), tail (33-35 *vs* 58-62 μm) and spicules (70-72 *vs* 107 μm), and fewer ventromedian supplements (three or four *vs* eight); from *S. pseudoventrale* by its smaller general size (L = 2.79-3.13 *vs* 3.72-5.92 mm, neck = 699-722 *vs* 850-1072 μm), longer odontostyle (14-15 *vs ca* 8.5 μm on its ventral side) and uterus (201-244 *vs* 123 μm long or 2.3-2.8 *vs* 1.3 times body diam.), shorter tail (33-35 *vs* 46-63 μm) and spicules (70-72 *vs* 90-95 μm), and fewer ventromedian supplements (three or four *vs* six or seven); and from *S. vietnamense* by its wider lip region (21-22 *vs* 14-16 μm) offset by constriction *vs* depression, and *pars refringens vaginae* present *vs* absent.

Sectonema ciliatum sp. n. also resembles *S. anisonchum* and *S. basilgoodeyi*, but can be distinguished from both by its smaller body (L = 2.79-3.13 *vs* >3.90 mm), and the presence of cilia-like structures in the anterior cheilostom. Moreover, it differs from *S. anisonchum* by its longer (14-15 *vs* 10.0-10.5 μm long on its ventral side) odontostyle (*vs* a mural tooth), shorter neck (699-722 *vs* 960-1040 μm) and pharyngeal expansion (60 *vs* 70% of total neck length), more anterior vulva (V = 52-53 *vs* 54-58), comparatively longer tail (c = 82-92 *vs* 100-125), shorter spicules (70-72 *vs* 84-96 μm), longer lateral guiding pieces (19-20 *vs* 13-14 μm), and fewer ventromedian supplements (three or four *vs* seven or eight); and from *S. basilgoodeyi* by its less slender body (a = 34-41 *vs* 49-53), shorter neck (699-722 *vs* 874-924 μm), uterus shorter (201-244 *vs ca* 366 μm or 2.3-2.8 *vs* 3.4 body diam. long) and a simple tube-like structure *vs* apparently tripartite, and more anterior vulva (V = 52-53 *vs* 58), shorter tail (33-35 *vs* 55 μm) and spicules (70-72 *vs* 110 μm), and fewer ventromedian supplements (three or four *vs* eight).

MOLECULAR CHARACTERISATION

Two 750-bp sequences of the D2-D3 of 28S rRNA gene were obtained from one female and one male. Both sequences were identical. The evolutionary relationships of the new species with several representatives of the order Dorylaimida are presented in Figures 9 and 10. The molecular data and their derived phylogenetic trees show similar phylogenetic relationships and topology to those obtained in previous studies (Álvarez-Ortega *et al.*, 2013a, b; Nguyen, *et al.*, 2016). The two *S. ciliatum* sp. n. sequences clustered together with its congeners, very close to *S. tropicum* Nguyen, Abolafia, Bonkowski, Peña-Santiago & Álvarez-Ortega, 2016, another species of the genus from the same geographical origin (Vietnam), in a well-supported clade (posterior probabilities: BI = 100 and ML = 98).

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References

Abolafia, J. & Peña-Santiago, R. (2005). Nematodes of the order Rhabditida from Andalucía Oriental: *Pseudacrobeles elongatus* (de Man, 1880) comb. n. *Nematology* 7, 917-926.

Altherr, E. (1958). Nématodes du bassin inférieur de la Weser et des dunes d'Héligoland: espèces nouvelles ou incomplètement décrites. *Mémoires de la Société Vaudoise des Sciences Naturelles* 12, 45-63.

Álvarez-Ortega, S., Subbotin, S.A. & Peña-Santiago, R. (2013a). Morphological and molecular characterisation of Californian species of the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae). *Nematology* 15, 431-439.

Álvarez-Ortega, S., Subbotin, S.A. & Peña-Santiago, R. (2013b). Morphological and molecular characterisation of Californian species of the genus *Metaporcelaimus* Lordello, 1965 (Dorylaimida, Aporcelaimidae), with a new concept of the genus. *Nematology* 15, 251-278.

Álvarez-Ortega, S., Nguyen, T.A.D., Vu, T.T.T., Abolafia, J. & Peña-Santiago, R. (2015). Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), and an updated taxonomy of the genus. *ZooKeys* 516, 1-26.

Baermann, G. (1917). Eine einfache Methode zur Auffindung von *Ankylostomum* (Nematoden) Larven in Erdproben. *Geneeskunding Tijdschrift voor Nederlandsch-Indië* 57, 131-137.

Coomans, A. & Loof, P.A.A. (1986). Observations on the glands of the male reproductive system in dorylaims and its phylogenetic importance. *Revue de Nématologie* 9, 261-265.

Flegg, J.J.M. (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology* 60, 429-437.

Heyns, J. (1965). On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimoid nematodes. *Entomology Memoirs, Department of Agricultural Technical Services, Republic of South Africa* 10, 1-51.

Holterman, M., Rybarczyk, K., van den Elsen, S., van Megen, H., Mooyman, P., Peña-Santiago, R., Bongers, T., Bakker, J. & Helder, J. (2008). A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* 8, 23-34.

Huelsenbeck, J.P. & Ronquist, F. (2001). MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754-755.

Loof, P.A.A. & Coomans, A. (1970). On the development and location of the oesophageal gland nuclei in Dorylaimina. In: *Proceedings of the IX International Nematology Symposium (Warsaw, Poland, 1967)*, pp. 79-161.

Nguyen, T.A.D., Abolafia, J., Bonkowski, M., Peña-Santiago, R. & Álvarez-Ortega, S. (2016). Two atypical new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. *European Journal of Taxonomy* 171, 1-20.

Nicholas, K.B., Nicholas Jr, H.B. & Deerfield II, D.W. (1997). GeneDoc: analysis and visualization of genetic variation. *EMBnet News* 4, 1-14.

Nylander, J.A.A. (2004). MrModeltest v2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala.

Peña-Santiago, R. & Álvarez-Ortega, S. (2014a). Studies on the genus *Sectonema* Thorne, 1930 (Dorylaimida: Aporcelaimidae). Redescription of *S. ventrale*, the type species of the genus. *Nematology* 16, 1097-1104.

Peña-Santiago, R. & Álvarez-Ortega, S. (2014b). Redescription of three species of the genus *Sectonema* Thorne, 1930 (Nematoda: Dorylaimida: Aporcelaimidae) originally studied by E. Altherr. *Zootaxa* 3881, 63-74.

Peña-Santiago, R., Abolafia, J. & Álvarez-Ortega, S. (2014). New proposal for a detailed description of the dorylaim spicule (Nematoda: Dorylaimida). *Nematology* 16, 1091-1095.

Ronquist, F. & Huelsenbeck, J. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.

Siddiqi, M.R. (1964). Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 2, 174-184.

Siddiqi, M.R. (1984). *Sectonema anisonchum* sp. n., *S. mucrodens* sp. n. and *S. truxum* sp. n. (Nematoda: Dorylaimida) from Colombian rain forest. *Indian Journal of Nematology* 14, 84-88.

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. (2006). Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455-474.

Swofford, D.L. (2003). *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b 10.* Sunderland, MA, USA, Sinauer Associates.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725-2729.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876-4882.

Thorne, G. (1930). Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. *Journal of Agricultural Research, USDA* 41, 445-466.

Publication 5

Re-description of *Cephalobus topali* Andrássy, 1970 (Rhabditida, Cephalobidae) from Vietnam, and transfer to Acrobeloides (Cobb, 1924) Thorne, 1937

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Re-description of *Cephalobus topali* Andrássy, 1970 (Rhabditida, Cephalobidae) from Vietnam, and transfer to *Acrobeloides* (Cobb, 1924) Thorne, 1937

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Abstract

Recently, specimens of *Cephalobus topali*, collected from an agricultural area from northern Vietnam, are described, including SEM study. They are characterized by their its 306–347 µm long body in females, lateral field with two alae or 3-4 longitudinal incisures, lip region with three pairs of asymmetrical conoid lips, three low and rounded labial probolae separate from the corresponding pair of lips by an incisure, U-shaped primary axils without guard processes, V-shaped secondary axils without guard processes, pharyngeal corpus slightly fusiform and 2.6–3.1 times the isthmus length, spermatheca and postvulval sac poorly developed, female tail conoid (18–24 µm, c = 12.7–17.0, c' = 1.8–2.7) with rounded terminus ending in an acute or wart-shaped mucro, phasmid at 29–34% of tail length, and male unknown. SEM pictures reveal that lip region pattern significantly differs from that found in members of *Cephalobus* and fits that observed in *Acrobeloides,* so that the species is transferred to this genus. *Cephalobus quadrilineatus* is provisionally maintained as different from *C. topali.*

Key words: *Acrobeloides*, cephalobids, *Cephalobus*, morphology, new combination, rhabditids, SEM, taxonomy

Introduction

The order Rhabditida Chitwood, 1933 has been studied for more than a century in many regions of the world, especially in temperate areas. However, for many areas there is still little information on the order. Knowledge of rhabditid taxa from Vietnam is limited to the work of Andrássy (1970), who recorded and described six species, namely *Ablechroiulus dudichi* Andrássy, 1970, *Cephalobus topali* Andrássy, 1970, *Heterocephalobus loofi* Andrássy, 1968, *Heteropleuronema unum* Andrássy, 1970, *Panagrocephalus baloghi* Andrássy, 1968 and *Stomachorhabditis vietnamicus* Andrássy, 1970.

Several nematological surveys were conducted in Vietnam in the last few years and yielded some rhabditid specimens, which will be studied in the future. Six females and eight larvae of *C. topali* were collected, a species only known to occur in Vietnam and originally described on the base of one female. An attempt was made to re-examine this female, but, unfortunately, the material had deteriorated so much that this was not possible. Here we present a re-description of the species, expanding its taxonomic characterization.

Material and methods

Extraction and processing of nematodes. Nematodes were collected from soil samples taken alongside the Pia Oac Nature Reserve, Cao Bang Province, Vietnam, extracted using the Baermann's funnel (1917) technique and Flegg's (1967) sieving methods, relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerol following De Grisse's (1969) technique, and mounted on permanent glass slides.

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Light microscopy (LM). Drawings were made using a drawing tube (*camera lucida*) attached to a Leitz Laborlux S microscope. Measurements were taken using an ocular micrometer and/or a curvimeter from drawings of the corresponding organ or structure. Micrographs were taken with a Nikon Eclipse 80i light microscope provided with differential interference contrast optics (DIC) and a Nikon Digital Sight DS-U1 camera. Micrographs were edited using Adobe® Photoshop® CS.

Scanning electron microscopy (SEM). A specimen preserved in glycerol was selected for observation using SEM following the protocol of Abolafia (2015). The nematode was hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5kv).

The terminology used for the morphology of stoma and spicules follows the proposals of De Ley *et al*. (1995) and Abolafia & Peña-Santiago (2006), respectively.

Taxonomic results

Cephalobus topali Andrássy, 1970

(Figs 1–3)

Material examined. Six females and eight larvae from Pia Oac, Vietnam, in acceptable state of preservation.

Morphometrics. See Table 1.

Description. *Female*: Body 0.31–0.35 mm long. Habitus straight or sigmoid after fixation. Cuticle distinctly annulated, annuli 2 mm thick at mid-body. Lateral field occupying 15–24% of mid-body diameter, with two alae (three or four longitudinal incisures, depending on either alae are slightly separate or not) arising at pharyngeal corpus level and fading away at phasmid level, appearing only one incisure posteriorly. With SEM, anterior region seen to comprise three pairs of asymmetrical conoid lips, and three low and rounded labial probolae separated from the corresponding pair of lips by an incisure; primary axils deep, U-shaped, lacking guard processes; secondary axils shallow, V-shaped, lacking guard processes. Sensillae papilliform, six labial and four cephalic. Amphids slit-like, with thickened margin. Stoma cephaloboid: cheilostom with rounded rhabdia; gymnostom narrower than cheilostom, and as wide as stegostom, the latter with small rhabdia. Pharynx also cephaloboid: slightly fusiform corpus, 2.6–3.1 times the isthmus length, with procorpus longer than metacorpus; isthmus narrower than corpus; basal bulb pyriform, bearing well developed valves at its middle. Cardia hemispheroid, surrounded by intestinal tissue. Intestine lacking specialization; its lumen often sigmoid. Nerve ring located at 56–76% of neck (stoma + pharynx) length, at level of the posterior part of corpus, near its junction to isthmus. Excretory pore at 61–87% of neck length, 44–48 annuli from the anterior end, at level of corpus-isthmus junction, in front of hemizonid. Deirid at 63–81% of neck length, 49–53 annuli from the anterior end, at level of basal bulb. Reproductive system monodelphic-prodelphic: ovary posteriorly directed, with or without a double flexure posterior to vulva level; oviduct short, difficult to distinguish; spermatheca poorly developed, 0.3–0.4 times the corresponding body diameter in length; uterus tube-like, as long as the corresponding body diameter, consisting of a shorter proximal part with thin walls and distinct lumen and a longer distal section having thick walls and small lumen; post-vulval sac 0.2–0.4 times the corresponding body diameter in length; vagina extending inwards to one-third of body diameter. Rectum 1.3–1.7 anal body widths in length. Tail conoid, with 11–13 annuli on the ventral side; terminus rounded, ending in an acute or wart-shaped mucro. Phasmid situated at 29–34% of tail length.

Male: Unknown.

Diagnosis (based on the 6 specimens studied). This species is characterized by its body length (306–347 μm in females), lateral field with two alae or 3–4 longitudinal incisures, lip region with three pairs of asymmetrical conoid lips, three low and rounded labial probolae separate from the corresponding pair of lips by an incisure, U-shaped primary axils without guard processes, V-shaped secondary axils without guard processes, pharyngeal corpus slightly fusiform and 2.6–3.1 times the isthmus length, spermatheca and post-vulval sac poorly developed, female tail conoid (18–24 μm, c = 12.7–17.0, c' = 1.8–2.7) with rounded terminus ending in an acute or wart-shaped mucro, and phasmid at 29–34% of tail length. Males are unknown.

Locality and habitat. Pia Oac (GPS coordinates: 22º36'28"N and 105º52'15"E, elevation 1150 m), Cao Bang Province, Vietnam, collected from the rhizosphere of *Zea mays* L. growing in an area of intensive agricultural crops alongside the Pia Oac Natural Reserve.

TABLE 1. Morphometrics of *Cephalobus quadrilineatus* Eroshenko, 1968 and *C. topali* Andrássy, 1970. Measurements in µm and in the form: mean ± standard deviation (range) where appropriate.

Species	*C. topali*		*C. quadrilineatus*
Locality/District	Pia Oac	Yen So, Hanoi	Pogranichny and Khankaysky
Province	Cao Bang	Hanoi	Primorsky Krai
Country	Vietnam	Vietnam	Russia
Habitat	Maize	Grass	Wheat
Reference	Present paper	Andrássy (1970)	Eroshenko (1968)
n	6♀♀	♀	13♀♀
Body length	317.7 ± 14.4 (306–345)	310	310–387
a	17.6 ± 1.4 (15.9–19.2)	17.0	13.8–18.7
b	3.1 ± 0.2 (2.9–3.3)	3.1	3.2–3.7
c	14.4 ± 1.5 (12.7–17.0)	15.0	13.0–14.7
c'	2.1 ± 0.3 (1.8–2.7)	1.7	2.1*
V	64.3 ± 1.4 (62–65)	65	61–68
Labial probolae	1.0 ± 0.1 (1.0-1.2)	1.4*	?
Lip region width	6.0 ± 0.6 (5–6)	7	?
Stoma length	10.5 ± 0.6 (10–11)	13	?
Pharyngeal corpus	56.5 ± 3.0 (51–59)	62*	?
Isthmus	20.0 ± 1.6 (18–22)	21*	?
Bulbus	15.2 ± 2.3 (14–20)	14	?
Pharynx length	94.0 ± 5.0 (83–96)	97*	?
Nerve ring - anterior end	76.0 ± 7.4 (60–77)	67*	?
Excretory pore - anterior end	68.0 ± 7.3 (57–72)	58*	?
Deirid - anterior end	75.0 ± 6.0 (65–78)	71**	?
Neck length	104.5 ± 5.4 (93–107)	105*	95**
Cardia	4.5 ± 0.8 (4–6)	4*	?
Annuli	1.0 ± 0.2 (1.0-1.4)	1.3	?
Cuticle at midbody	0.8 ± 0.0 (0.8)	0.8	?
Body diameter at neck base	18.5 ± 1.5 (16–20)	20**	?
Body diameter at midbody	18.5 ± 1.9 (16–20)	18**	22
Lateral field width	4.0 ± 0.5 (3–4)	3*	?
Anterior ovary	58.0 ± 16.9 (57–96)	108*	?
Spermatheca	6.5 ± 0.6 (6–7)	0?	?
Anterior uterus length	19.5 ± 2.6 (18–24)	47*	?
Postvulvar sac	6.5 ± 1.3 (4–7)	8*	0
Vagina length	5.0 ± 0.0 (5)	4*	?
Vulva - anterior end	201.8 ± 11.8 (189–224)	210**	192**
Rectum length	15.0 ± 1.9 (14–19)	13**	?
Anal body diameter	10.5 ± 1.5 (9–13)	13**	?
Tail length	23.0 ± 2.3 (18–24)	22	25**
Phasmid - anus distance	8.5 ± 1.0 (7–9)	9**	?

* Measurements from drawings, ** from other measurements.

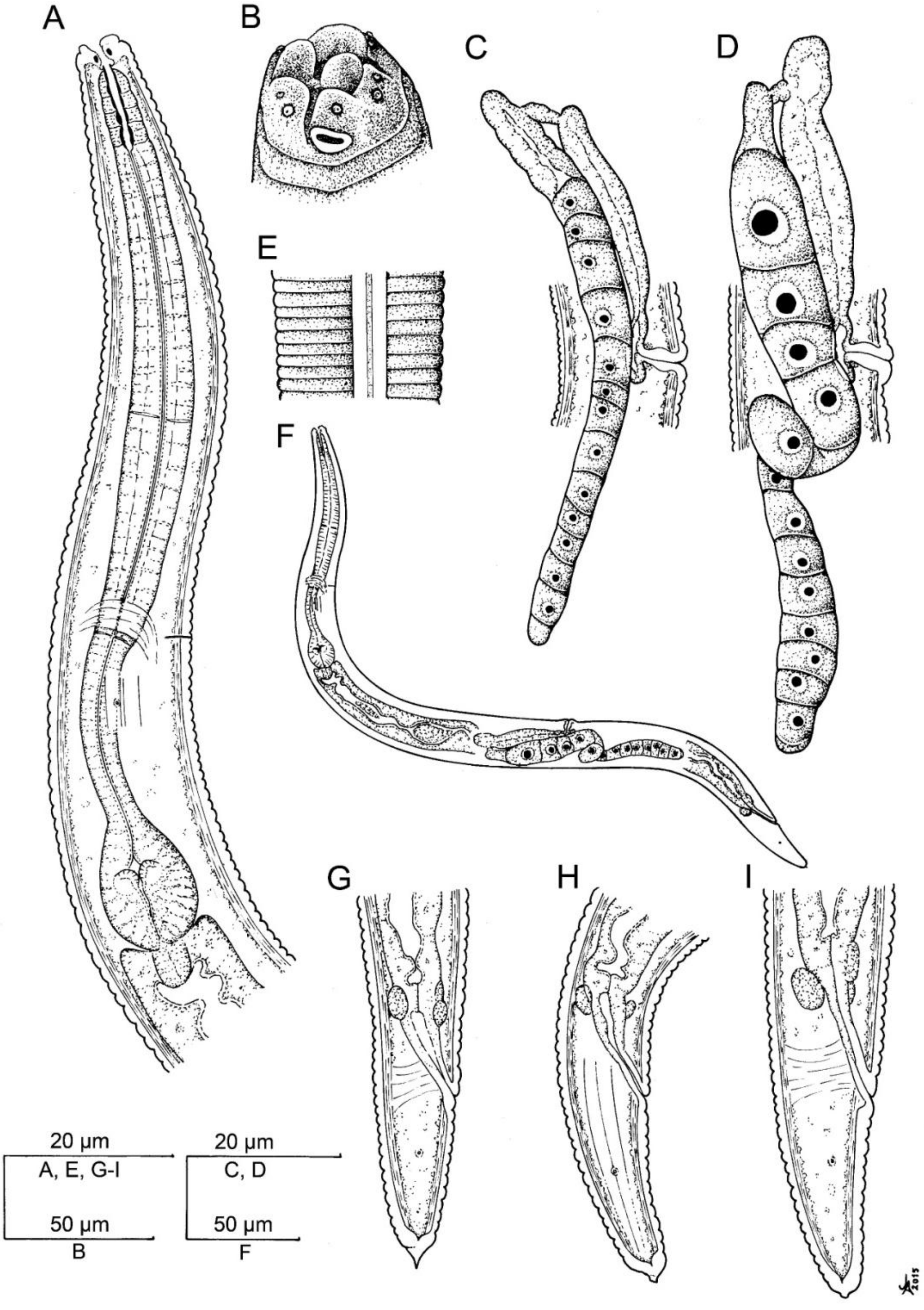

FIGURE 1. *Acrobeloides topali* (Andrássy, 1970) **n. comb.** (female, in lateral view). A: Anterior end. B: Lip region. C, D: Reproductive system. E: Lateral field. F: Entire female. G–I: Posterior end.

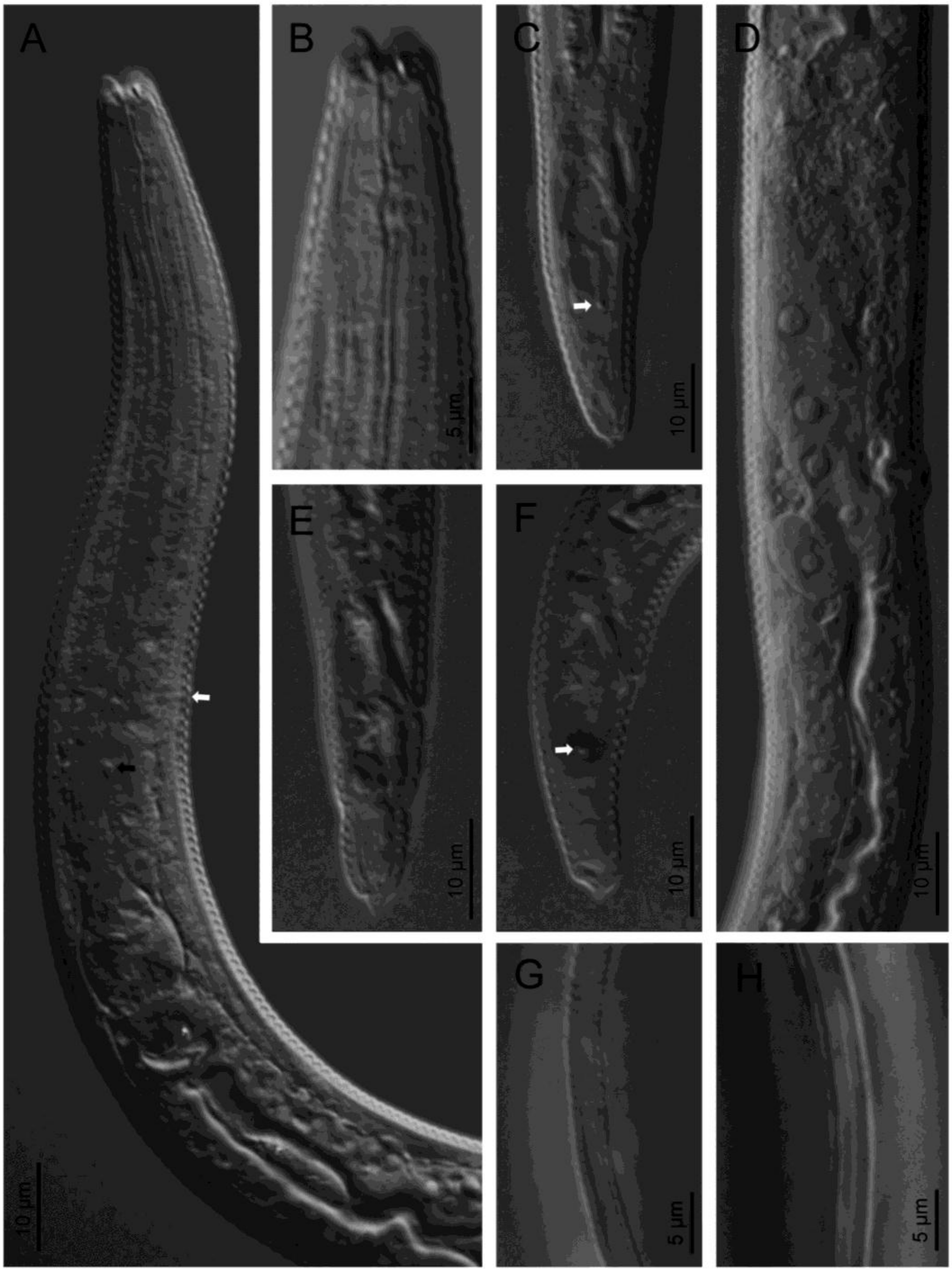

FIGURE 2. *Acrobeloides topali* (Andrássy, 1970) **n. comb.** (female, light micrographs). A: Anterior end (white arrow indicates the excretory pore, black arrow pointing at the deirid). B: Stoma. C: Hemizonid (arrowed). D: Reproductive system. E, F, H: Tail (arrows pointing at the phasmid). G: Deirid (arrowed). I, J: Lateral field.

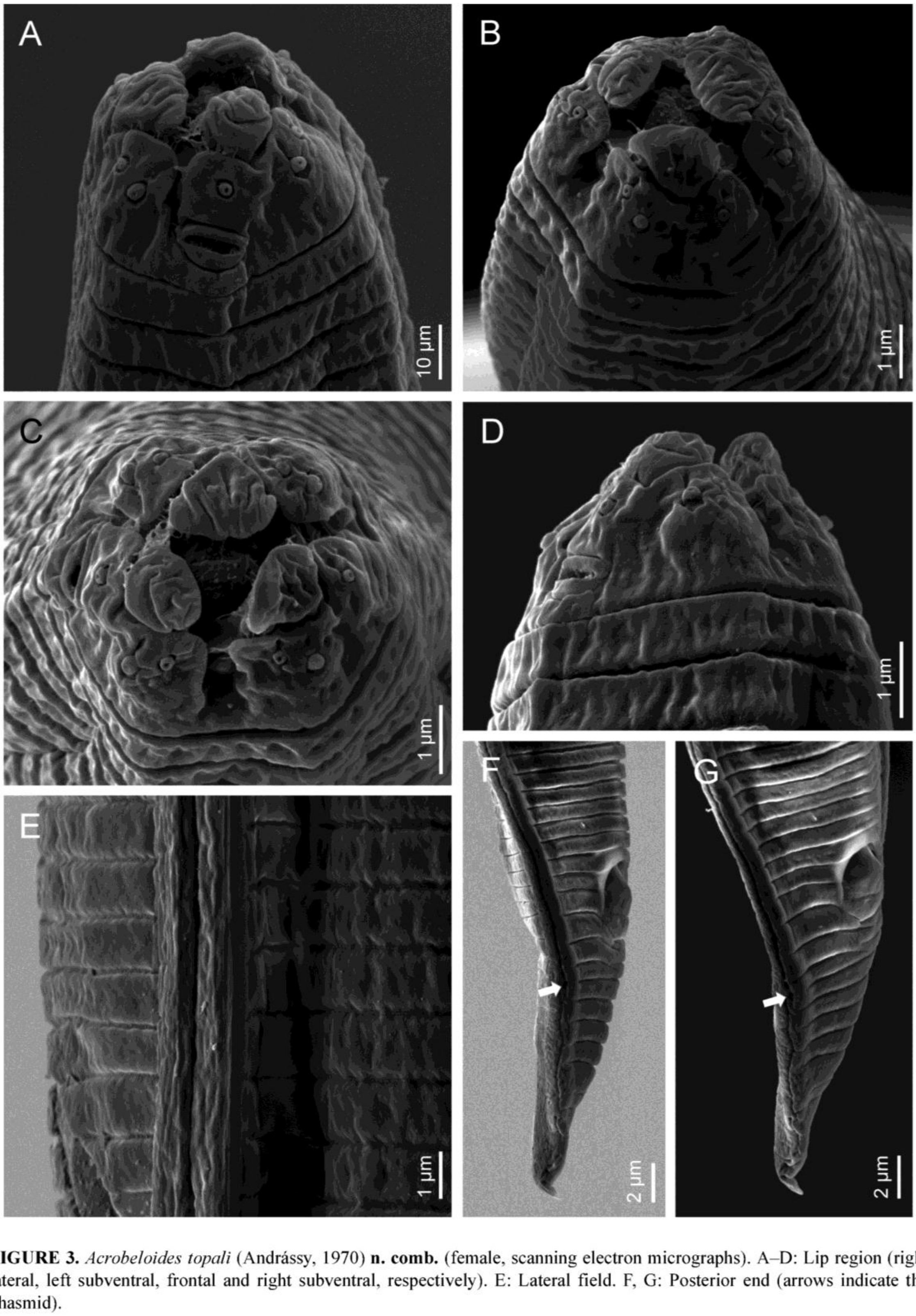

FIGURE 3. *Acrobeloides topali* (Andrássy, 1970) **n. comb.** (female, scanning electron micrographs). A–D: Lip region (right lateral, left subventral, frontal and right subventral, respectively). E: Lateral field. F, G: Posterior end (arrows indicate the phasmid).

Discussion. *Cephalobus topali* was originally described from only one female and three larval specimens. The material herein studied perfectly fits Andrássy's (1970) description. The SEM observations reported here provide additional information that raises doubt about the position of this species. For example, instead of connecting the corresponding adjacent pairs of lips, a typical diagnostic feature of the genus *Cephalobus* Bastian, 1865 (see Abolafia & Peña-Santiago 2009), the labial probolae of the specimen examined here are clearly separated from the lips by an incisures (Fig. 3A–C). This fits the pattern observed in representatives of the genus *Acrobeloides* (Cobb, 1924) Thorne, 1937 (see Abolafia & Peña-Santiago, 2003). Consequently, this species is transferred to the genus *Acrobeloides* as *A. topali* (Andrássy, 1970) **comb n.**

Andrássy (1984) regarded *C. topali* as identical to and a junior synonym of *Cephalobus quadrilineatus* Eroshenko, 1968, only known to occur in Russian Far East Territories. Both species have similar morphology and morphometry (see comparison in Table 1), and both have an acute mucro at the tail tip. Later, however, Andrássy (2005) maintained both species as valid, but examination of specimens of the Russian species is necessary to confirm it. Provisionally, we accept that the two species are valid as differences between them were noted: isthmus size (as long as *vs* one-third of the corresponding body diameter), excretory pore location (at level of the corpus-isthmus junction *vs* at isthmus posterior portion, near the basal bulb), presence of a short *vs* absence of a post-vulval sac, and rectum length (as long as *vs* shorter than the corresponding body diameter).

The distribution of *A. topali* appears to be restricted to the north of Vietnam, associated with modified soils. Andrássy (1970) found it among grass roots in the municipal cemetery of the district of Yen So (Hanoi), and here it is reported from further north, in agricultural plots opened in the rain forest, close to the Pia Oac Natural Reserve. In the last two years, several hundred specimens of nematodes have been examined from this area but, only a few specimens of this rare species were found, and none came from natural, unaltered areas.

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References

Abolafia, J. (2015) A low-cost technique to manufacture a container to process meiofauna for scanning electron microscopy. *Microscopy Research and Technique*, 78, 771–776.
http://dx.doi.org/10.1002/jemt.22538

Abolafia, J. & Peña-Santiago, R. (2003) Nematodes of the order Rhabditida from Andalucía Oriental, Spain. The genus *Acrobeloides* (Cobb, 1924) Thorne, 1937, with description of *A. arenicola* sp. n. and a key to species. *Journal of Nematode Morphology and Systematics*, 5 (2002), 107–130.

Abolafia, J. & Peña-Santiago, R. (2006) Nematodes of the order Rhabditida from Andalucía, Spain. The family Panagrolaimidae, with a compendium of species of *Panagrolaimus* and a key to their identification. *Journal of Nematode Morphology and Systematics*, 8, 133–159.

Abolafia, J. & Peña-Santiago, R. (2009) Nematodes of the order Rhabditida from Andalucía Oriental, Spain. The genus *Cephalobus* Bastian, 1865 with description of *C. harpagonis* sp. n. and key to species. *Nematology*, 11, 485–508.
http://dx.doi.org/10.1163/138855409X12465362560359

Andrássy, I. (1968) Fauna Paraguayensis. 2. Nematoden aus den Galeriewäldern des Acaray-Flusses. *Opuscula Zoologica Budapest*, 8, 167–312.

Andrássy, I. (1970) Freilebende Nematoden aus Vietnam. *Opuscula Zoologica Budapestinensis*, 10, 5–31.

Andrássy, I. (1984) *Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida). Bestimmungsbücher zur Bodenfauna Europas. No. 9*. Berlin, Deutschland, Akademie Verlag, 509 pp.

Andrássy, I. (2005) *Free-living nematodes of Hungary (Nematoda errantia). Vol. 1. In:* Csuzdi, C. & Mahunka, S. (Eds.), *Pedozoologica Hungarica. No. 3*. Hungarian Natural History Museum, Budapest, pp. 1–518.

Baermann, G. (1917) Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben.

Geneeskunding Tijdschrift voor Nederlandsch-Indië, 57, 131–137.

Bastian, H.C. (1865) Monograph on the Anguillulidae, or free nematoids, marine, land, and freshwater; with descriptions of 100 new species. *Transactions of the Linnean Society of London - Zoology*, 25, 73–184. http://dx.doi.org/10.1111/j.1096-3642.1865.tb00179.x

Chitwood, B.G. (1933) A revised classification of the Nematoda. *Journal of Parasitology*, 20, 1–131.

Cobb, N.A. (1924) Amended characterization of the nemic genera *Cephalobus* and *Acrobeles*. *Journal of Parasitology*, 11, 108.

De Grisse, A. (1969) Redescription ou modifications de quelques techniques utililisées dans l'etude des nematodes phytoparasitaires. *Mededelingen van de Rijksfaculteit Landbouwwetenschappen Gent*, 34, 351–369.

De Ley, P., van de Velde, M.C., Mounport, D., Baujard, P. & Coomans, A. (1995) Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). *Nematologica*, 41, 153–182. http://dx.doi.org/10.1163/003925995X00143

Eroshenko, A.S. (1968) [*Cephalobus quadrilineatus* n. sp. – new species of nematode of the genus *Cephalobus* Bastian, 1865 (Nematoda: Cephalobidae)] in Russian. *Soobshchenija Dalnevostotjnogo Filiala Akademii Nauk SSSR*, 26, 55–57.

Flegg, J.J.M. (1967) Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology*, 60, 429–437. http://dx.doi.org/10.1111/j.1744-7348.1967.tb04497.x

Thorne, G. (1937) A revision of the nematode family Cephalobidae Chitwood and Chitwood, 1934. *Proceedings of the helminthological Society of Washington*, 4, 1–16.

Publication 6

Two new species of the genus *Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam

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Two new species of the genus *Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam

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Abstract

Two new species of *Aporcelinus* from Vietnamese natural habitats are studied, described and illustrated, including line drawings and light microscope (LM) pictures. *Aporcelinus paramamillatus* **sp. n.** is characterized by its 1.71–2.14 mm long body, lip region 21–22 μm broad, odontostyle 22–24 μm long at its ventral side, neck 451–503 μm long, uterus tripartite and 71–94 μm long, $V = 53–54$, tail conical with finely rounded tip, ending in a short peg-like terminus, occasionally slightly re-curved dorsad (24–34 μm, $c = 50–77$, $c' = 0.6–0.9$) and unknown male. *Aporcelinus paraseychellensis* **sp. n.** is distinguished by its 1.20–1.46 mm long body, lip region 16–18 μm broad, odontostyle 20 μm long at its ventral side, neck 354–368 μm long, uterus tripartite and 176–241 μm long, $V = 52–54$, tail conical with acute tip and hardly re-curved dorsad, spicules 62 μm long, and 12 regularly spaced ventromedian supplements lacking hiatus.

Key words: *Aporcelinus,* description, morphology, new species, taxonomy, Vietnam

Introduction

Andrássy (2009a) erected the genus *Aporcelinus* to accommodate two new species, *A. altitudinalis* and *A. mediterraneus*, and seven previously known taxa that were transferred from *Allodorylaimus* Andrássy, 1986, *Aporcelaimellus* Heyns, 1965, and *Eudorylaimus* Andrássy, 1959. A few other species were later added by Andrássy (2009b, 2012), Álvarez-Ortega & Peña-Santiago (2011, 2013) and Vinciguerra *et al.* (2014), bringing the total number of species to 18. *Aporcelinus* was originally classified under Aporcelaimidae Heyns, 1965, Aporcelaimellinae Andrássy, 2009, but also compared to *Allodorylaimus* in Qudsianematidae Jairajpuri, 1965, Qudsianematinae Jairajpuri, 1965. It was separated from the aporcelaimid genera *Aporcelaimellus* and *Makatinus* Heyns, 1965 by its thinner cuticle at tail (*vs* distinctly thickened), absence (*vs* presence) of cervical lacunae, presence (*vs* absence) of a dorsal lobe at the pharyngo-intestinal junction, absence (*vs* presence) of a hiatus, and conical (*vs* mostly short and rounded) tail with pointed (*vs* rounded) tip; and from *Allodorylaimus* by its three-layered (*vs* two-layered) cuticle, aporcelaimoid (*vs* typical dorylaimoid) guiding apparatus and tail not curved ventrad (*vs* distinctly curved ventrad). More recently, Vinciguerra *et al.* (2014) raised some doubt about the classification of *Aporcelinus* under Aporcelaimidae due to features also shared with members of Qudsianematidae.

Abundant material of *Aporcelinus* was collected in natural and cultivated areas of Vietnam in several nematological surveys conducted during the last three years. Detailed studies revealed that this material might contain several unknown species. The description of two of them is presented in the following paper. This contribution is part of a series of papers (see Vu *et al.*, 2010; Álvarez-Ortega *et al.*, 2015) devoted to explore the diversity of Vietnamese dorylaims.

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Material and methods

Sampling. Soil samples were collected from natural areas of Vietnam and temporarily stored in plastic bags. Nematodes were extracted using Baermann's (1917) and Flegg's (1967) methods, relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerine following Siddiqi's (1964) technique, and mounted on permanent glass slides to allow handling and observation under the light microscope.

Light microscopy. Specimens were measured, drawn and identified under light microscopes. Morphometrics include Demanian ratios and other measurements. The location of the pharyngeal gland nuclei is expressed according to Loof & Coomans (1970) and spicule terminology follows Peña-Santiago *et al*. (2014). Line illustrations were made with an Olympus BX51 light microscope equipped with a series of objectives (4x, 10x, 20x, 40x, and 100x oil immersion), differential interference contrast optics (DIC) and drawing tube. Drawings were made using a *camera lucida*.

Microphotographs were taken with a Nikon Eclipse 80i light microscope provided with DIC and a Nikon Digital Sight DS-U1 camera. Raw photographs were edited using Adobe® Photoshop® CS.

Taxonomic results

***Aporcelinus paramamillatus* sp. n.**

(Figs 1–2)

Material examined. Six females from one location, in good condition.

Morphometrics. See Table 1.

TABLE 1. Morphometrics of *Aporcelinus paramamilatus* sp. n. Measurements in µm (except L, in mm), and in the form: mean ± standard deviation (range).

Locality		Cat Ba National Park (Hai Phong Province)		
Habitat		*Cinnamomum* sp.		Maize
Character	n	Holotype (♀)	Paratypes (4♀♀)	♀
L		1.71	1.98 ± 0.20 (1.71–2.14)	1.86
a		25	23.4 ± 1.7 (21.1–25.1)	26
b		3.8	4.1 ± 0.3 (3.8–4.5)	4.1
c		55	64.7 ± 13.2 (50.2–76.8)	77
c'		0.8	0.7 ± 0.1 (0.6–0.9)	0.6
V		54	53.4 ± 0.5 (52.9–53.9)	54
Lip region diam.		21	22 ± 0.5 (21–22)	21
Odontostyle length at ventral side		24	23.5 ± 1.0 (22–24)	24
Odontophore length		45	48.5 ± 1.9 (45–49)	45
Neck length		451	474.0 ± 21.3 (451–503)	457
Pharyngeal expansion length		183	239.0 ± 13.3 (219–251)	234
Diam. at neck base		67	74 ± 4.6 (70–81)	69
at midbody		69	84.5 ± 7.5 (75–92)	71
at anus		39	42.5 ± 3.9 (39–47)	37
Prerectum length		73	82.5 ± 3.3 (77–84)	80
Rectum length		41	48 ± 3.0 (44–51)	44
Tail length		31	31.5 ± 3.6 (27–34)	24

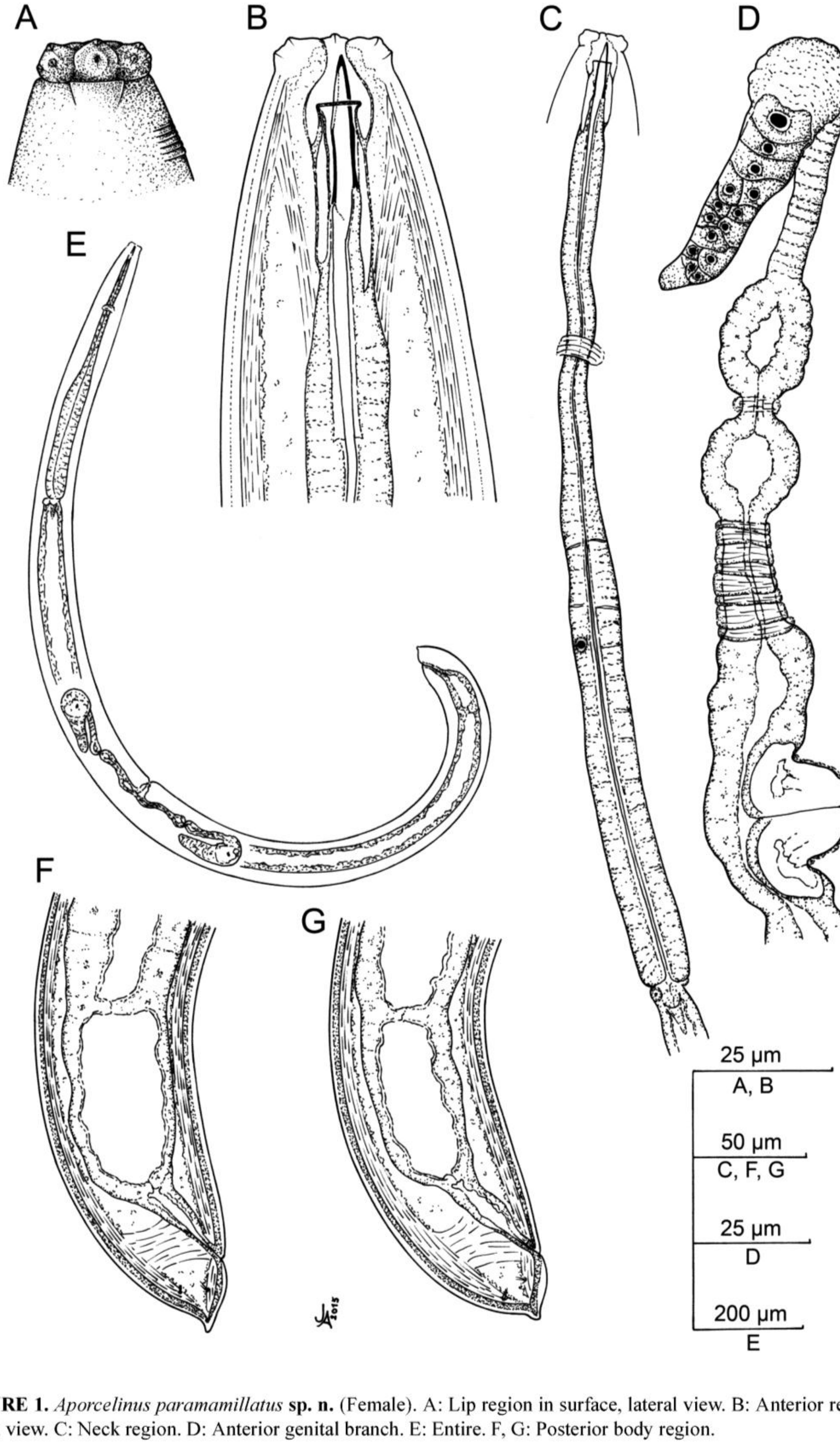

FIGURE 1. *Aporcelinus paramamillatus* **sp. n.** (Female). A: Lip region in surface, lateral view. B: Anterior region in median, lateral view. C: Neck region. D: Anterior genital branch. E: Entire. F, G: Posterior body region.

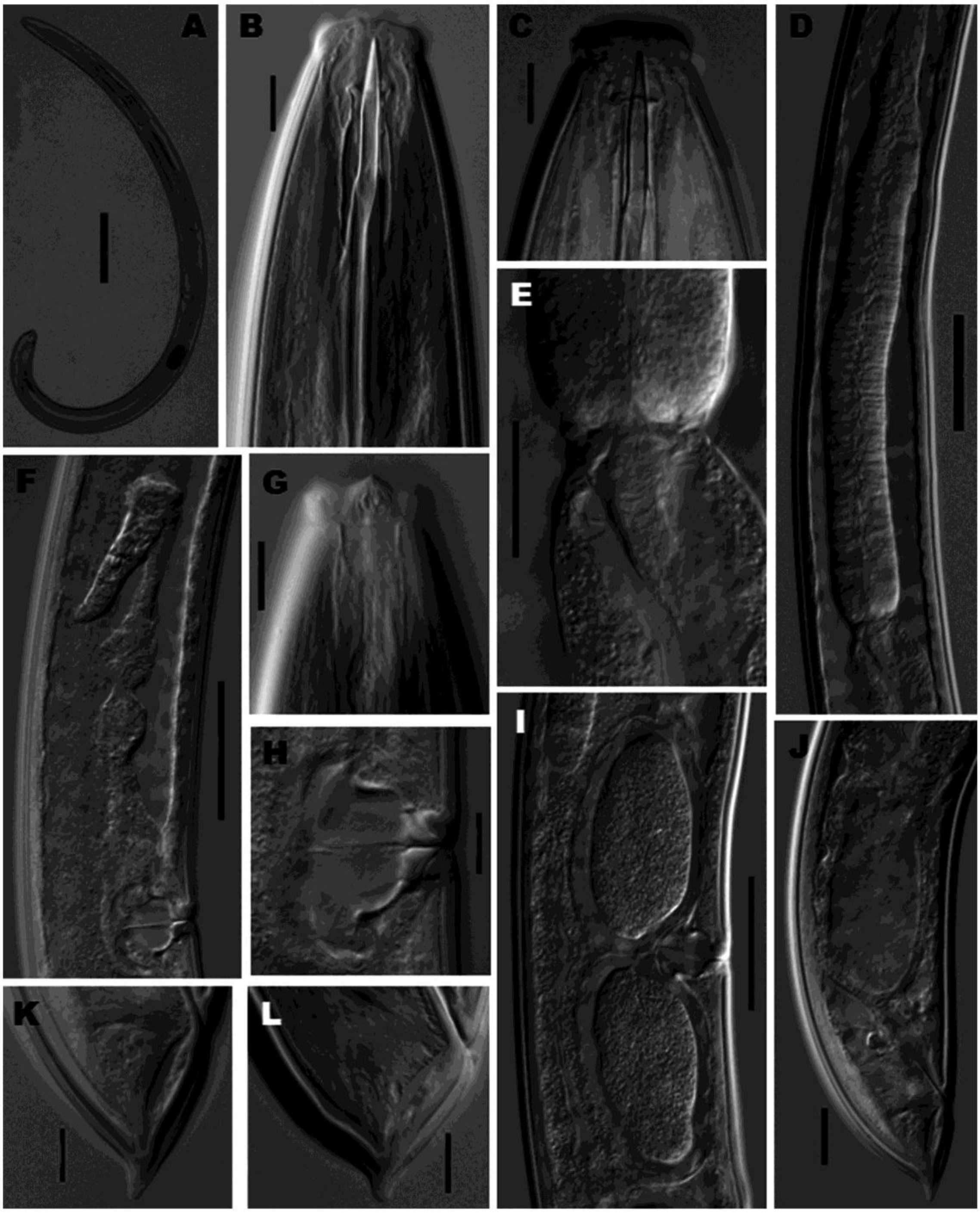

FIGURE 2. *Aporcelinus paramamillatus* **sp. n.** (Female, LM). A: Entire. B, C: Anterior region in lateral, median view. D: Pharyngeal expansion. E: Pharyngo-intestinal junction. F: Anterior genital branch. G: Anterior region in lateral, surface view. H: Vagina. I: Uterine eggs. J: Posterior body region. K, L: Caudal region. (Scale bars: A = 200 μm; B, C, G, H, K, L = 10 μm; D, F, I = 50 μm; E, J = 20 μm.)

Description. *Female:* Moderately slender (a = 21–26) nematodes of medium size, 1.71–2.14 mm long. Habitus visibly curved ventrad upon fixation, C-shaped. Cuticle two-layered, 4 µm thick at anterior region and mid-body and 4–7 µm on tail; inner layer thicker and more refractive than the outer layer. Lateral chords 10–14 µm broad or 12–19% of mid-body diameter. Body pores often obscure, but two cervical dorsal and two ventral are sometimes distinct at the level of the odontophore. Lip region somewhat angular, offset by weak constriction, 2.6–2.8 times as wide as high and 26–31% of body diameter at neck base; lips moderately separated, with protruding labial and cephalic papillae. Amphid fovea cup-shaped, its opening 10–12 µm broad or about one-half of lip region diameter. Cheilostom nearly as long as wide, with no specialization. Odontostyle strong, slightly (1.1–1.2 times) longer than lip region diameter, 5.2–6.5 times as long as wide and 1.1–1.5% of total body length; aperture 12 µm or 46–50% of its total length. Guiding ring simple but distinct and somewhat plicate, located at 12–13 µm or 0.5 times the lip region diameter from the anterior end. Odontophore rod-like, 1.7–2.0 times the odontostyle in length. Pharynx consisting of a slender but muscular anterior section enlarging very gradually into the posterior expansion that is 6.4–8.3 times as long as wide, 2.7–3.4 times the corresponding body diameter long, and occupies 47–56% of total neck length; gland nuclei located as follows (n = 1): DO = 58, DN = 62, S_1N_1 = 75, S_1N_2 = 79, S_2N = 92. Nerve ring at 163–212 µm or 31–35% of total neck length from the anterior end. Pharyngo-intestinal junction bearing a distinctly developed dorsal lobe; cardia conical, 12–18 x 8–15 µm. Genital system didelphic-amphidelphic, with both branches almost equally and moderately developed, 157–224 µm long or 9–12% of total body length: ovaries 57–177 µm long, surpassing the oviduct-uterus junction; oviduct 65–106 µm long or 0.9–1.4 times the body diameter, and consisting of a slender portion and a moderately developed *pars dilatata*; a narrowing separates oviduct and uterus; uterus 71–94 µm long or 1.0–1.6 times the corresponding body diameter long, tripartite, consisting of a short and wider proximal region, a narrower intermediate section with no lumen, and a nearly spherical distal part; uterine egg 90 x 55 µm, with irregular shell; vagina extending inwards 26–32 µm or 32–43% of body diameter, with *pars proximalis* 16–22 x 18–22 µm and somewhat sigmoid walls surrounded by weak musculature, *pars refringens* consisting of two drop-shaped pieces 6–7 x 4–6 µm and a combined width of 9–12 µm, and *pars distalis* 3–5 µm long and visibly refractive; vulva a transverse slit. Prerectum 1.8–2.2, rectum 1.0–1.2 times the anal body diameter long. Tail conical with finely rounded tip, dorsally and ventrally convex, ending in a short peg-like terminus, occasionally slightly re-curved dorsad; hyaline portion very short; caudal pores two pairs at the anterior part of tail, one subdorsal, the other sublateral.

Male: Unknown.

Diagnosis. The new species is characterized by its 1.71–2.14 mm long body, lip region offset by weak constriction and 21–22 µm broad, odontostyle 22–24 µm at its ventral side or 1.1–1.2 times the lip region diameter, neck 451–503 µm long, pharyngeal expansion 183–251 µm long or 46–56% of total neck length, uterus tripartite and 71–94 µm long or 1.0–1.6 times the corresponding body diameter long, V = 53–54, tail conical (27–34 µm, c = 50–77, c' = 0.6–0.9) ending in a peg-like terminal portion and not visibly re-curved dorsad, and male absent.

Relationships. This species is very similar to *A. mamillatus* (Williams, 1959) Álvarez-Ortega & Peña-Santiago, 2011 in its general morphology and many morphometrics, but it can be distinguished by several significant differences: broader lip region (21–22 *vs* 19–20 µm), smaller odontostyle (22–24 *vs* 25–29 µm, 1.1–1.2 *vs* 1.3–1.5 times the lip region diameter, n=9) with shorter aperture (46–50 *vs* 57–61% of its total length), more posterior S_2N (92 *vs* 83–86) and more posterior vulva (V = 53–54 *vs* V = 49–52).

Type locality and habitat. Northern Vietnam, Cat Ba National Park, Hai Phong Province (GPS coordinates: 20° 47'45" N and 107° 00'40"E, elevation 170 m), in soil of a secondary forest with *Cinnamomum* sp. as the dominant plant species. One female was collected near the same location in the rhizosphere of *Zea mays* L. growing in an area of intensive agricultural crops alongside the National Park.

Type material. Female holotype and four females paratypes deposited in the nematode collection of the University of Jaén, Spain. One female paratype in the nematode collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Etymology. The specific epithet means 'near *mamillatus*' as the new species resembles *A. mamillatus* in many aspects.

Aporcelinus paraseychellensis **sp. n.**

(Figs 3–5)

Syn. *Aporcelinus seychellensis apud* Vinciguerra *et al.* (2014), *nec* Andrássy (2009)

Material examined. Three females and one male from two locations, in acceptable condition.

Morphometrics. See Table 2.

TABLE 2. Morphometrics of *Aporcelinus paraseychellensis* sp. n. Measurements in µm (except L, in mm).

Locality		Cat Ba National Park (Hai Phong Province)			Huu Lien Natural Reserve (Lang Son Province)
Habitat		*Cinnamomum* sp			*Machilus* sp. and *Dimocarpus* sp.
		Holotype	Paratypes		
Character	n	♀	♀	♂	♀
L		1.46	1.31	1.40	1.19
a		17	18	20	16
b		4.1	3.7	3.8	3.3
c		45	38	33	34
c'		0.9	1.0	1.2	0.9
V/T		54	52	53	53
Lip region diam.		17	18	16	17
Odontostyle length at ventral side		20	20	20	20
Odontophore length		36	37	–	35
Neck length		354	354	368	363
Pharyngeal expansion length		175	175	186	184
Diam. at neck base		77	66	65	56
at midbody		88	74	69	73
at anus		35	34	35	37
Prerectum length		72	59	80	54
Rectum length		46	42	60	43
Tail length		33	34	42	35
Spicule length		–	–	62	–
Ventromedian supplements		–	–	12	–

Description. *Adult:* Stout (*a* = 16–20) nematodes of medium size, 1.20–1.46 mm long. Habitus variably curved ventrad upon fixation, C-shaped in female, G-shaped in male. Cuticle two-layered, 3–4 µm thick at anterior region, 3.5–5.0 µm in mid-body and 4.0–6.5 µm on tail; inner layer thicker and more refractive than the outer layer. Lateral chord 9 µm broad or 10–13% of mid-body diameter. Body pores often obscure, but two cervical pores are visible on both dorsal and ventral sides at level of odontostyle plus odontophore. Lip region angular, offset by constriction; lips high, separated, with protruding labial and cephalic papillae. Amphid fovea cup-shaped, its opening 9–11 µm broad or 53–63% of lip region diameter. Cheilostom nearly as long as wide, with no specialization. Odontostyle strong, 1.1–1.4 times longer than lip region diameter, 6.2–7.7 times as long as wide and 1.4–1.9% of total body length; aperture 10–11 µm or 46–48% of its total length. Guiding ring simple but distinct, somewhat plicate, located at 9–10 µm or 0.5–0.6 times the lip region diameter from the anterior end. Odontophore rod-like, 1.5–1.8 times the odontostyle length. Pharynx consisting of a slender but muscular anterior section enlarging very gradually in the posterior expansion, which is 5.4–6.8 times as long as wide, 2.8–3.3 times the corresponding body diameter and occupies 49–51% of total neck length; gland nuclei located as follows (n = 1): DO = 58, DN = 62, S_1N_1 = 75, S_1N_2 = 82, S_2N = 90. Nerve ring at 113 µm or 31% of total neck length from the anterior end. Pharyngo-intestinal junction bearing a distinctly developed dorsal lobe; cardia conical, 10–14 x 11–12 µm. A dorsal cell mass is present at level of the pharyngo-intestinal junction.

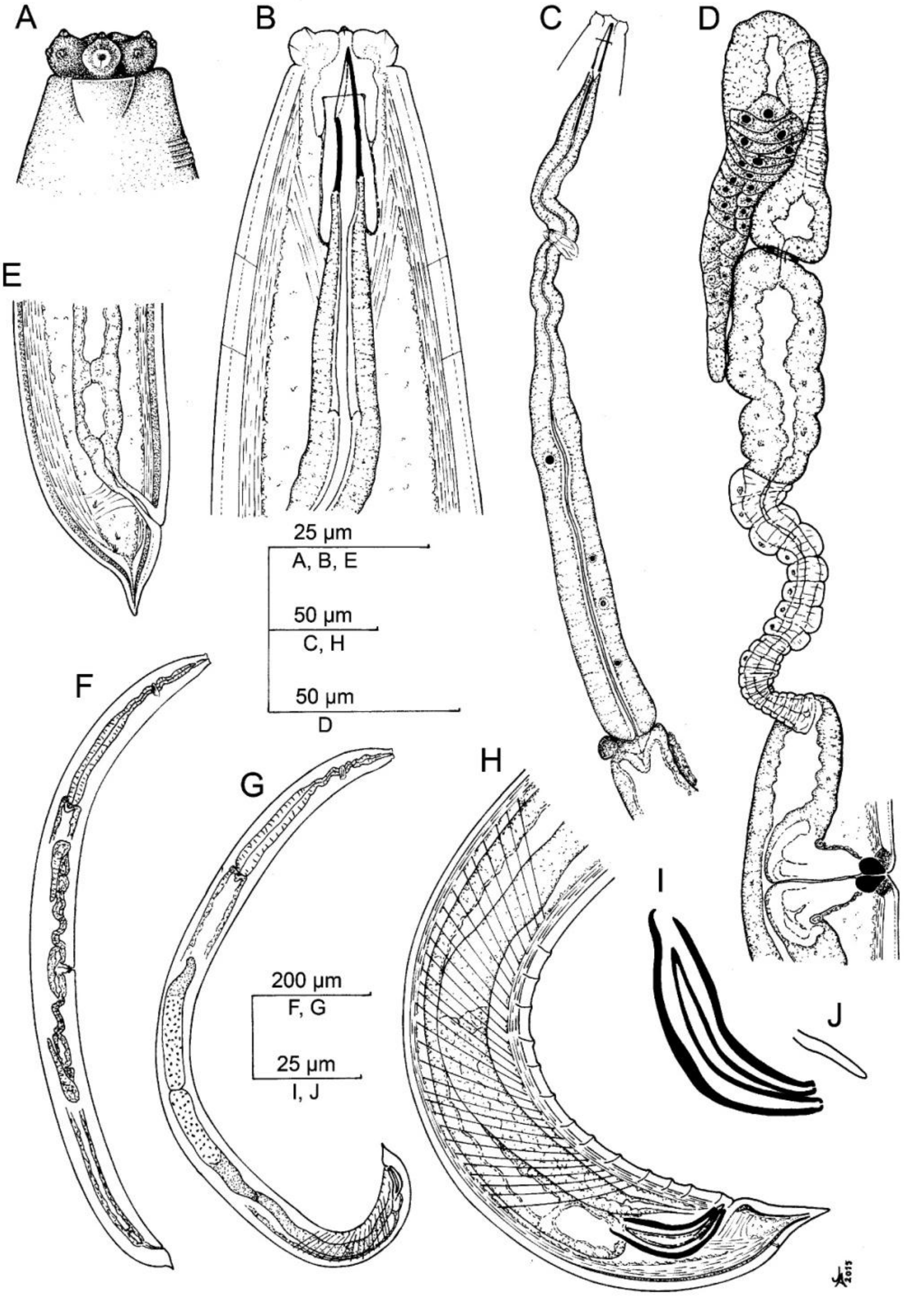

FIGURE 3. *Aporcelinus paraseychellensis* **sp. n.** A: Lip region in surface, lateral view. B: Anterior region in median, lateral view. C: Neck region. D: Female, anterior genital branch. E: Female, posterior body region. F: Female, entire. G: Male, entire. H: Male, posterior body region. I: Spicule. J: Lateral guiding piece.

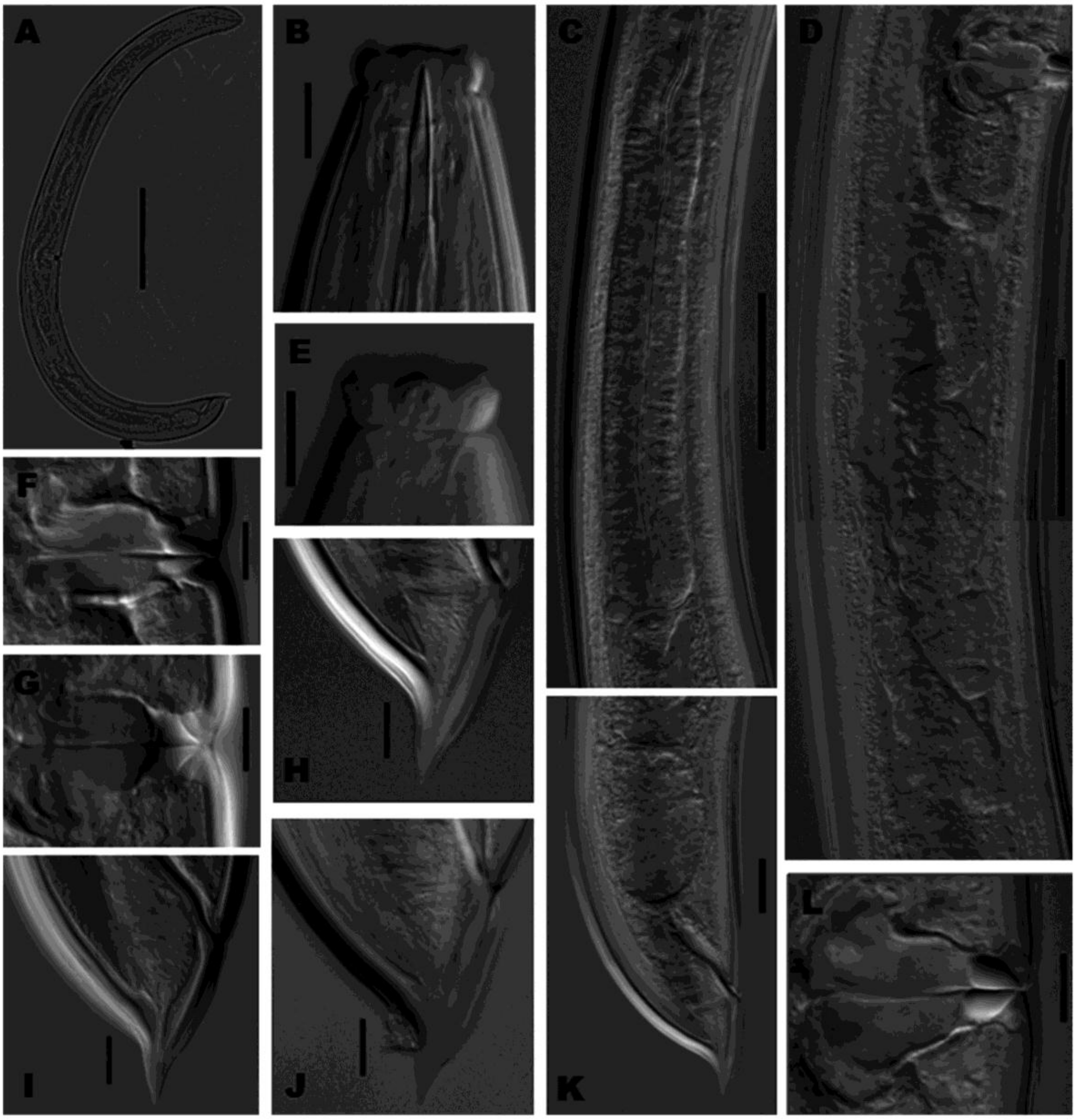

FIGURE 4. *Aporcelinus paraseychellensis* **sp. n.** (Female, LM). A: Entire. B: Anterior region in lateral median view. C: Pharyngeal expansion and pharyngo-intestinal junction. D: Posterior genital branch. E: Lip region in lateral surface view. F, G, L: Vagina. H–J: Caudal region. K: Posterior body region . (Scale bars: A = 200 µm, B, E–J, H, L = 10 µm; C, D = 50 µm; K = 20 µm.)

Female: Genital system didelphic-amphidelphic, with both branches equally and moderately developed, 176–308 µm long or 17–21% of total body length: ovaries very variably sized, 79–236 µm long; oviduct 55–94 µm long or 0.8–1.1 times the body diameter and consisting of a slender portion made of prismatic cells and a moderately developed *pars dilatata* with perceptible lumen; a narrowing separates oviduct and uterus, but a distinct sphincter is lacking; uterus 176–241 µm long or 2.4–2.7 times the corresponding body diameter, tripartite, consisting of a short and wider proximal region, a longer and much narrower intermediate section with no lumen, and a nearly spherical distal part; vagina extending inwards 33–36 µm or 40–47% of body diameter, with *pars proximalis* 22–25 x 15–20 µm and somewhat sigmoid walls surrounded by weak musculature, *pars refringens* consisting of two drop-shaped or trapezoidal pieces 7–8 x 4–5 µm and a combined width of 9–10 µm, and *pars* *distalis* 5–7 µm long and visibly refractive; vulva a transverse slit. Prerectum 1.5–2.0, rectum 1.2–1.3 times the anal body diameter in length. Tail conical with acute tip, ventrally nearly straight or slightly convex, and dorsally first convex and then concave, the tail being slightly re-curved dorsad; cuticle extending until the tail tip, so that a hyaline portion is not observed; caudal pores two pairs, at the middle of tail, one subdorsal, the other lateral.

FIGURE 5. *Aporcelinus paraseychellensis* **sp. n.** (Male, LM) A: Entire. B: Pharyngo-intestinal junction. C: Pharyngeal expansion. D: Male, posterior body region. E, F: Spicule. G: Caudal region. (Scale bars: A = 200 µm; B = 20 µm, C, D = 50 µm; E–G = 10 µm.)

Male: Prerectum 2.3, cloaca 1.7 times the body diameter at the cloacal aperture. Genital system diorchic, with opposite testes. In addition to the adcloacal pair, located at 9 µm from the cloacal aperture, there is a series of 12 low, regularly spaced (10–16 µm apart) ventromedian supplements, the most posterior three located within the range of spicules. Spicules dorylaimid, 4.6 times longer than wide and 1.8 times longer than body diameter at the cloacal aperture: dorsal side regularly convex and ventral side with barely perceptible hump and hollow; curvature 136°; head 8 x 7 µm, with the dorsal side somewhat longer than the ventral one and weakly curved; median piece occupying less than one-third (29%) of maximum spicule width, reaching the posterior end of the spicule and with a visibly furcate tip; posterior end of spicules 6 µm wide. Lateral guiding pieces 17 µm long or 6.1 times as long as wide, slightly sigmoid and tapering posteriorly. Tail straight ventrally, dorsally first convex and then very weakly concave.

Diagnosis. The new species is characterized by its 1.20–1.46 mm long body, lip region offset by constriction and 16–18 µm broad, odontostyle 20 µm at its ventral side or 1.1–1.4 times the lip region diameter, neck 354–368 µm long, pharyngeal expansion 175–186 µm long or 49–51% of total neck length, a dorsal cell mass present at the pharyngo-intestinal junction, uterus tripartite and 176–241 µm, or 2.4–2.7 times the corresponding body diameter, long, $V = 52–54$, tail conical with acute tip and slightly recurved dorsad (33–42 µm, $c = 33–45$, $c' = 0.9–1.2$), spicules 62 µm long, and 12 regularly spaced ventromedian supplements, three of them within the range of spicules.

Relationships. The new species is very similar to *A. seychellensis* Andrássy, 2011 in its general morphology and morphometrics, but differs from the type population of that species in its larger general size (body length 1.20–1.46 (n = 4) *vs* 0.92–1.07 mm (n=4); neck length 354–368 *vs* 272–330 µm), much larger genital branches (17–21% *vs* 7–13% of total body length each), more posterior vulva ($V = 52–54$ *vs* $V = 45–52$), shorter female tail (32–36 *vs* 36–40 µm, $c = 33–45$ *vs* $c = 25–29$) and male present (*vs* absent). Especially relevant in the type population are the very short female genital branches, probably because the uterus is short and simple.

Vinciguerra *et al.* (2014) studied two *A. seychellensis* populations from Ecuador that differ from the type populations in their larger general size [body 1.08–1.55 (n=26) *vs* 0.92–1.07 (n = 4) mm long] and comparatively shorter female tail ($c = 29–42$ *vs* $c = 25–29$). Besides, the female genital branches are larger (12–22 *vs* 7–13% of total body length), but lower values in the Ecuador material may be due to the convoluted, tripartite uterus. Leaving aside the fewer ventromedian supplements (7–9 *vs* 12; two *vs* three situated within the range of spicules) in males from Ecuador, there is no significant difference between this material and the Vietnamese specimens herein studied. Taking into consideration that only one male of the new species has been collected, the differences in number and arrangement of ventromedian supplements are provisionally regarded as intraspecific variability and, consequently, we consider the specimens from Ecuador and Vietnam to be conspecific.

Type locality and habitat. Northern Vietnam, Cat Ba National Park, Hai Phong Province (GPS coordinates: 20° 47'45" N and 107° 00'40"E, elevation 170 m), collected from soil in a tropical forest with *Cinnamomum* sp. as dominant plant species.

Other locality and habitat. Northern Vietnam, Huu Lien Natural Reserve, Lang Son Province, (GPS coordinates: 21° 42'51" N and 106° 21'48"E, elevation 502 m), in soil of the forest where the dominant plant species were *Machilus* sp. and *Dimocarpus* sp.

Type material. Female holotype, female paratype and male paratype deposited in the nematode collection of the University of Jaén, Spain. One female paratype deposited in the nematode collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Etymology. The specific epithet means 'near *seychellensis*' as the new species resembles *A. seychellensis* in many aspects.

Acknowledgements

The authors thank the directors of Cat Ba National Park (Hai Phong Province) and Huu Lien Natural Reserve (Lang Son Province) for issuing relevant permits to collect soil samples. This research was supported by the project entitled "Aporcelaimidae Mundi: Revisión de la familia Aporcelaimidae Heyns, 1965 (Nematoda, Dorylaimida)" (ref. CGL2012-33239; co-financed FEDER), the German Academic Exchange Service (DAAD) (PKZ 91540366), the Zoological Institute (University of Cologne), Germany and Idea-Wild, United States of America.

References

Álvarez-Ortega, S. & Peña-Santiago, R. (2011) Studies on the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae). Species from Sri Lanka originally studied by Loos. *Nematology*, 13, 193–209.
http://dx.doi.org/10.1163/138855410X513213

Álvarez-Ortega, S. & Peña-Santiago, R. (2013) Taxonomy of the genus *Aporcelaimellus* Heyns, 1965 (Nematoda, Dorylaimida, Aporcelaimidae). *Zootaxa*, 3669 (3), 243–260.
http://dx.doi.org/10.11646/zootaxa.3669.3.3

Álvarez-Ortega, S., Nguyen, T.A.D., Abolafia, J., Vu, T.T.T. & Peña-Santiago, R. (2015) Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), with an updated taxonomy of the genus. *Zookeys*, 516, 1–26.
http://dx.doi.org/10.3897/zookeys.516.10087

Andrássy, I. (1959) Taxonomische Uebersicht der Dorylaimen (Nematoda). I. *Acta Zoologica Academiae Scientiarum Hungaricae*, 5, 191–240.

Andrássy, I. (1986) The genus *Eudorylaimus* Andrássy, 1959 and the present status of its species (Nematoda: Qudsianematidae). *Opuscula Zoologica Budapestinensis*, 22, 1–42.

Andrássy, I. (2009a) *Aporcelinus*, a new genus of aporcelaimoid nematodes (Dorylaimida), and its species. *International Journal of Nematology*, 19, 121–136.

Andrássy, I. (2009b) Another species of the genus *Aporcelinus* Andrássy, 2009 (Nematoda: Dorylaimida). *Opuscula Zoologica Budapestinensis*, 40, 99–102.

Andrássy, I. (2011) Phylum Nematoda. *In:* Gerlach, J. (Ed.), *Crustacea, Platyhelminthes, Nematoda, Annelida and Tardigrada of the Seychelles Islands*: 15–57. Siri Scientific Press, Manchester. 160 pp.

Andrássy, I. (2012) Two new species of the family Aporcelaimidae (Nematoda. Dorylaimida). *Genus*, 23, 189–199.

Baermann, G. (1917) Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. *Geneeskunding Tijdschrift voor Nederlandsch-Indië*, 57, 131–137.

Flegg, J.J.M. (1967) Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology*, 60, 429–437.
http://dx.doi.org/10.1111/j.1744-7348.1967.tb04497.x

Heyns (1965) On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimid nematodes. *Entomology Memoirs Department of Agriculture Technical Services Republic of South Africa*, n° 10, 1–51.

Jairajpuri, M.S. (1965) *Qudsianema amabilis* n. gen., n. sp. (Nematoda: Dorylaimoidea) from India. *Proceedings of the Helminthological Society of Washington*, 32, 72–73.

Loof, P.A.A. & Coomans, A. (1970) On the development and location of the oesophageal gland nuclei in Dorylaimina. *Proceedings of the IX International Nematology Symposium (Warsaw, Poland, 1967)*, 79–161.

Peña-Santiago, R., Abolafia, J. & Álvarez-Ortega, S. (2014) New proposal for a detailed description of the dorylaim spicule (Nematoda: Dorylaimida). *Nematology*, 16, 1091–1095.
http://dx.doi.org/10.1163/15685411-00002834

Siddiqi, M.R. (1964) Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, 2, 174–184.

Vinciguerra, M.T., Orselli, L. & Clausi, M. (2014) One new and two known species of *Aporcelinus* Andrássy, 2009 and a new species of *Coomansinema* Ahmad & Jairajpuri, 1989 (Nematoda: Dorylaimida). *Nematology*, 16, 303–322.
http://dx.doi.org/10.1163/15685411-00002767

Vu, T.T., Ciobanu, M., Abolafia, J. & Peña-Santiago, R. (2010) Two remarkable new species of the genus *Crassolabium* Yeates, 1967 from Vietnam (Nematoda: Dorylaimida: Qudsianematidae). *Journal of Natural History*, 44, 2049–2064.
http://dx.doi.org/10.1080/00222933.2010.481055

Williams, J.R. (1959) Studies on the nematode soil fauna of sugarcane fields in Mauritius. 3. Dorylaimidae (Dorylaimoidea, Enoplida). *Mauritius Sugar Industry Research Institute Occasional Paper*, 3, 1–28.

Publication 7

Sectonema caobangense sp. n. from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae)

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***Sectonema caobangense* sp. n. from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae)**

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Abstract: Sectonema caobangense sp. n. from evergreen forest soil in Vietnam is described, including scanning electron micrograph (SEM) observations and D2-D3 LSU rDNA analysis. The new species is characterized by its 3.12 to 5.80 mm long body, lip region offset by deep constriction and 21 to 23 μm broad, mural tooth 13 to 14 μm long at its ventral side, 940 to 1,112 μm long neck, pharyngeal expansion occupying 61% to 69% of total neck length, uterus a long simple tube-like structure 292 to 363 μm long or 2.7 to 2.9 times the corresponding body diameter, *pars refringens vaginae* well developed, $V = 48$ to 56, short (36–51 μm, $c = 77–132$, $c' = 0.5–0.8$) and rounded tail, 87 to 99 μm long spicules, and four or five irregularly spaced ventromedian supplements bearing hiatus. *Sectonema caobangense* sp. n. differs from the typical pattern of *Sectonema* in the nature of the stomatal protrusible structure, bearing a mural tooth attached to the ventral side of the stoma. Molecular data obtained and the derived evolutionary trees support a close phylogenetic relationship with other *Sectonema* species.

Key words: Bayesian inference, description, LSU ribosomal DNA, maximum likelihood, morphology, morphometrics, SEM, taxonomy.

The genus *Sectonema* Thorne, 1930 is a remarkable, free-living nematode taxon, whose representatives are characterized, among other biological features, by their comparatively large size and predator behaviour (Heyns, 1965). It includes nearly 30 species from many countries and territories, mainly dwellers of terrestrial habitats. The taxonomy of the genus is somewhat intricate as its species are often separated by small differences, for instance the morphology of lip region, the nature of the stomatal protruding structure, tail shape, etc.

Several nematological surveys have revealed that Vietnamese nematode fauna is very rich in members of the family Aporcelaimidae Heyns, 1965, including many nondescribed species (for instance, see Álvarez-Ortega et al., 2015), some of them belonging to *Sectonema* (Nguyen et al., 2016). The study of a new population containing both females and males, collected from natural areas in the northern region of Vietnam, resulted in the discovery of an unknown species of *Sectonema*, which is described in the following. For additional introductory information, see the previous contributions already mentioned.

MATERIAL AND METHODS

Nematodes: Nematodes were collected from natural areas in Vietnam, extracted from soil samples using the methods of Baermann (1917) and Flegg (1967), relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerine following Siddiqi's (1964) technique, and mounted on permanent glass slides to allow handling and observation under light microscope (LM).

Light microscopy: Measurements of specimens were taken using a light Olympus BH-2 microscope equipped with differential interference contrast. Morphometrics included de Man's indices and usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof and Coomans (1970) and spicule terminology followed Peña-Santiago et al. (2014). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe Photoshop CS. Drawings were made using a *camera lucida*.

Scanning electron microscopy: After their examination and identification, two specimens preserved in glycerine were selected to their observation under SEM following the protocol by Abolafia and Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol–acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin scanning electron microscope.

DNA extraction, PCR, and sequencing: DNA was extracted from a single individual using the proteinase K protocol. Nematode material was transferred to an 0.5-ml Eppendorf tube containing 18 μl of worm lysis buffer (50 mM KCL, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40, and 0.45% Tween 20) and 2 μl proteinase K (600 μg/ml) (Thermo Scientific). The tubes were incubated at 65°C (1 hr) and then at 95°C (15 min). PCR and sequence protocols were as described in detail by Nguyen et al. (2016).

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The primers used for amplification of D2-D3 region of 28S rRNA gene were D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin et al., 2006). The sequence obtained was submitted to the GenBank database under accession number KX018821.

Phylogenetic analyses: The newly obtained sequences were aligned with other 48 D2-D3 expansion segments of 28S rRNA gene sequences available in GenBank using ClustalX 1.83 (Thompson et al., 1997). Outgroup taxa were chosen according to the results of previous published data (Holterman et al., 2008; Álvarez-Ortega et al., 2013). Sequence alignments were manually edited using GenDoc 2.6.002 (Nicholas et al., 1997). The sequence dataset was analysed with Bayesian inference (BI) and maximum likelihood (ML) using MrBayes

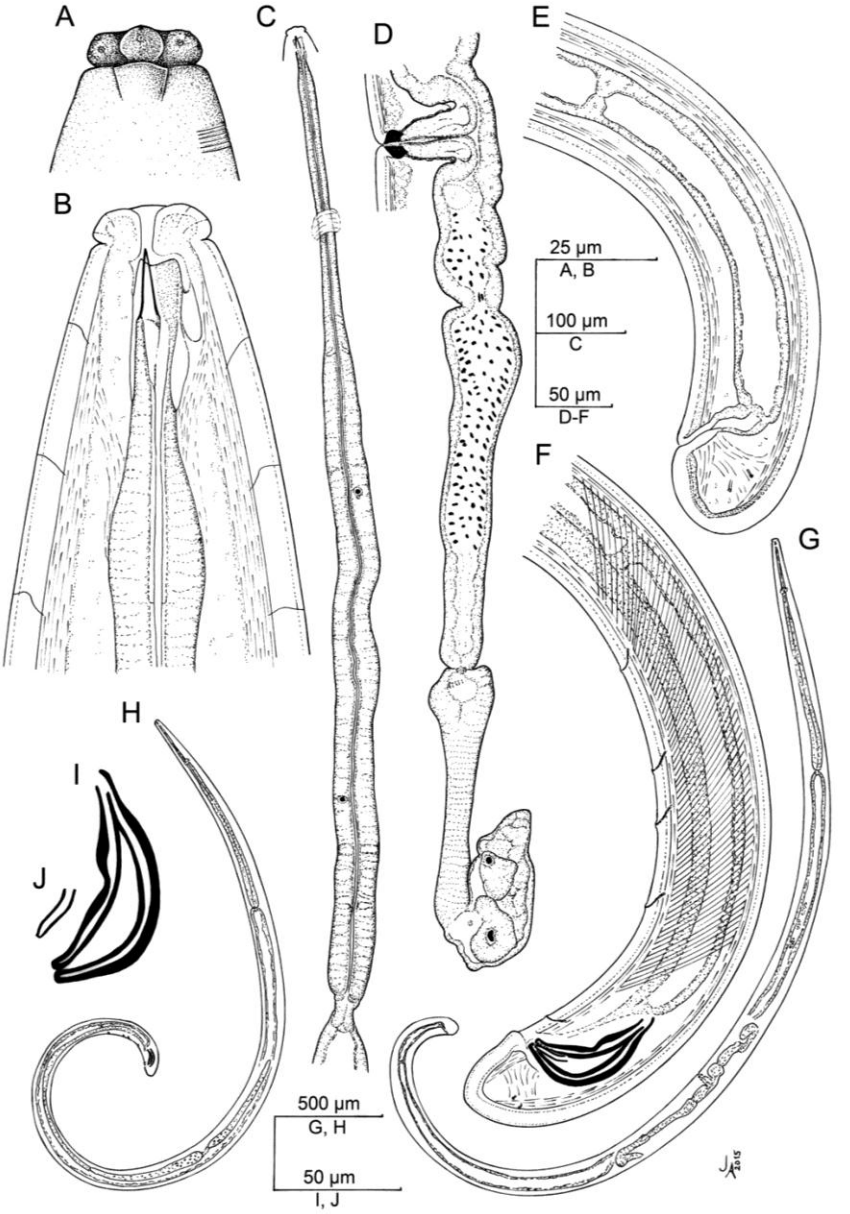

FIG. 1. *Sectonema caobangense* sp. n. (Line). A. Lip region in lateral surface view. B. Anterior region in mid-optical level, lateral view. C. Neck region. D. Female, posterior genital branch. E. Female, posterior body region. F. Male, posterior body region. G. Female, entire. H. Male, entire. I. Spicule. J. Lateral guiding piece.

3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and MEGA 6 (Tamura et al., 2013), respectively. The best-fit model of DNA evolution for BI was obtained using the program MrModeltest 2.3 (Nylander, 2004) with the Akaike Information Criterion in conjunction with PAUP* 4b10 (Swofford, 2003). Bayesian inference analysis under the GTR + G + I model was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo for 10^6 generations. Maximum likelihood analysis was implemented under the best-fitting evolutionary model (GTR + I + G), obtained using the program MEGA 6, and 1,000 bootstrap replications. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities are given on appropriate clades. The trees were visualized with the program FigTree v1.4.0 and drawn with Adobe Acrobat XI Pro 11.0.1.

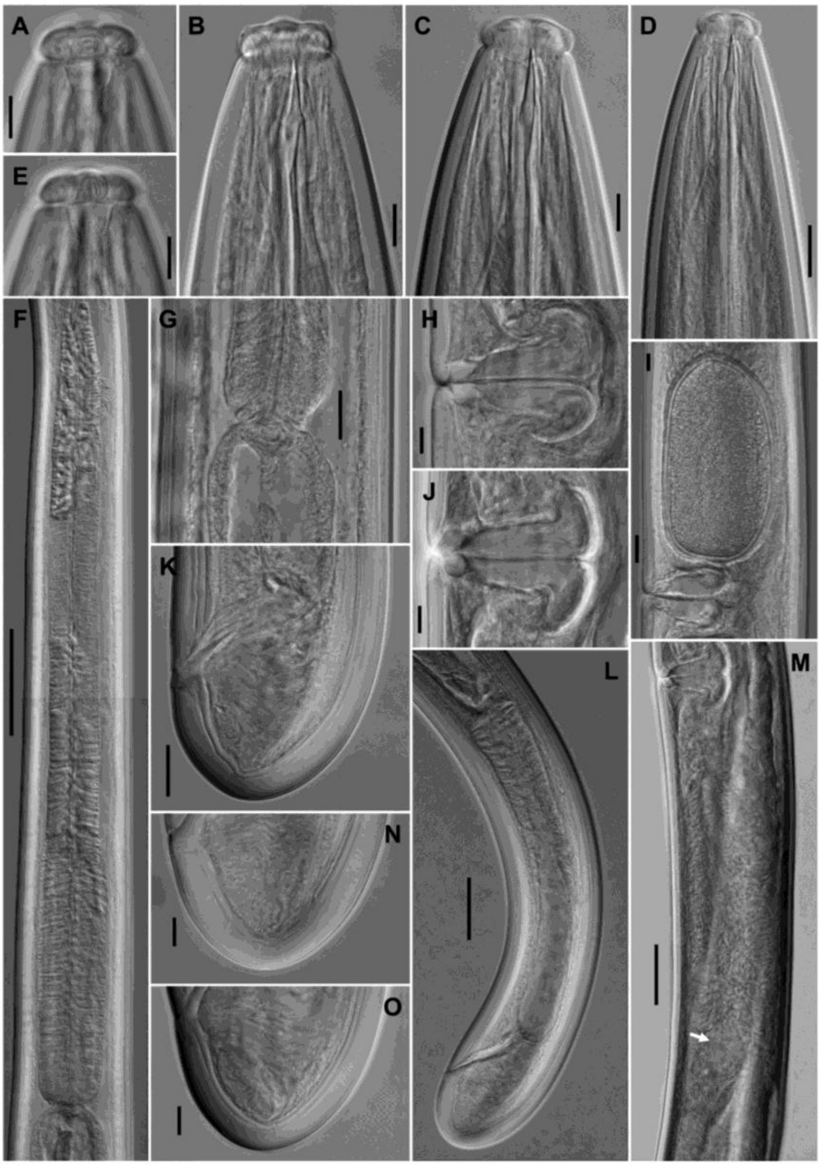

Fig. 2. *Sectonema caobangense* sp. n. (Female, LM). A, E. Lip region in lateral, surface view. B–D. Anterior region in lateral, median view. F. Pharyngeal expansion. G. Pharyngo-intestinal junction. H, J. Vagina. I. Vagina and uterine egg. K, N, O. Caudal region. L. Posterior body region. M. Posterior uterus, containing sperm cells inside. (Scale bars: A–C, E, H, J, N, O = 10 μm; D, G, I, K = 20 μm; F = 100 μm; L, M = 50 μm).

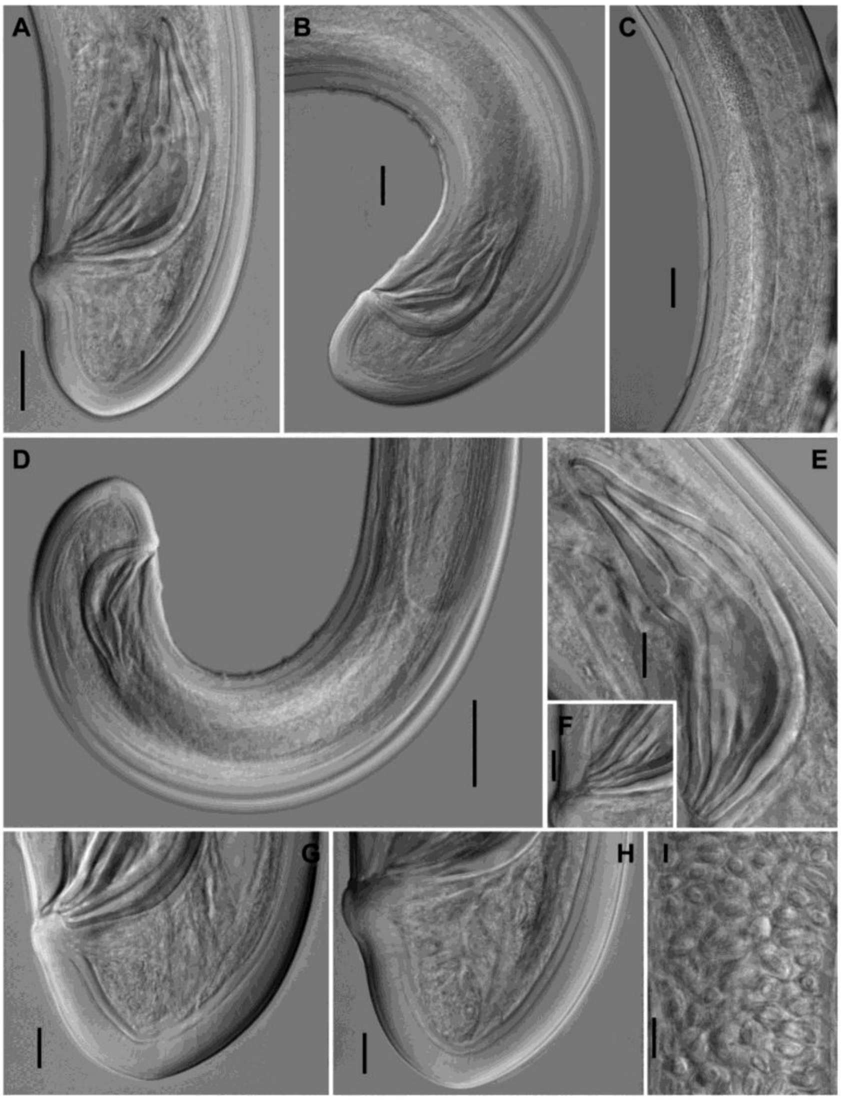

FIG. 3. *Sectonema caobangense* sp. n. (Male, LM). A. Spicule and caudal region. B, D. Posterior body region. C. Ventromedian supplements arrangement. E. Spicule. F. Lateral guiding piece. G, H. Caudal region. I. Sperm cells. (Scale bars: A–C = 20 µm; D = 50 µm; E–I = 10 µm).

RESULTS

Sectonema caobangense sp. n.
urn:lsid:zoobank.org:act:6C8D8A69-DF2B-481E-905A-913649522473

(Figs. 1-4; Table 1)

Material examined: Six females and six males from Vietnam, in variable state of preservation.

Description

Adult: Moderately slender to slender nematodes of big size, 4.02 to 5.80 mm long in females, 3.12 to 4.21 mm long in males. Body cylindrical, distinctly tapering toward the anterior end, less so toward the posterior end as the caudal region is rounded. Habitus regularly curved ventrad after fixation, C- or G-shaped, more curved at posterior body region in males, and occasionally spiral-like. Cuticle 4.0 to 4.5 µm thick at anterior region, 5 to 7 µm in mid-body and 8.5 to 12.0 µm on tail; three-layered, especially distinguishable at caudal region, where it consists of thinner outer layer bearing fine transverse striation through the entire body, thicker intermediate layer with radial striation, and thin inner layer. Lateral chord 10 to 18 µm wide at mid-body, occupying one-tenth to one-sixth (9–16%) of mid-body diameter. Two ventral and two dorsal body pores are usually present at level of odontophore. Lip region offset by deep constriction, 3.0 to 3.4 times as wide as high and less than one-fourth (19–23%) of body diameter at neck base; lips (under SEM) low, mostly amalgamated, each with four or five concentric striations; labial papillae, both inner and

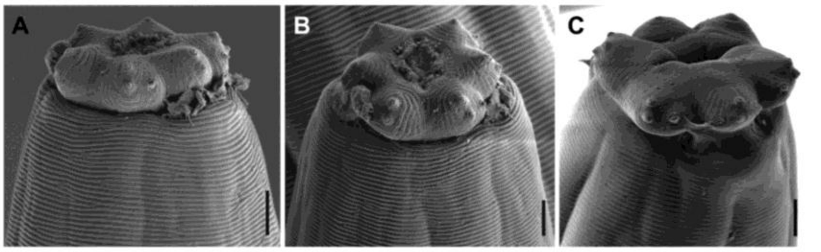

FIG. 4. *Sectonema caobangense* sp. n. (SEM). A. Juvenile, lip region in sublateral view. B. Juvenile, lip region in ventral view. C. Male, lip region in lateral view. (Scale bars: A–C = 3 μm).

outer, button-like, surrounded by a small ring-like incisure each, and bearing a pore at its center; cephalic papillae not surrounded by any ring-like incisures and with a short, transverse, slit-like opening; oral field visibly hexagonal, delimited by short radial incisures; oral aperture a large dorsoventral slit, the lip region hence showing bilateral symmetry. Amphideal fovea cup-shaped, its opening occupying 9 to 12 μm or two-fifths to three-fifths (43–59%) of lip region diameter. Cheilostom nearly cylindrical, lacking any differentiation. Mural tooth attached subventrally, 3.4 to 3.7 times longer than its maximum width, 0.6 times as long as lip region diameter and 0.24% to 0.43% of body length; dorsal side always distinctly longer than the ventral one and with more or less sigmoid in contour. Guiding ring simple, somewhat plicate, at 0.5 to 0.6 lip region diameters from anterior end. Odontophore linear, rod-like, 3.3 to 3.7 times the mural tooth length at its ventral side. Anterior region of pharynx enlarging very gradually; basal expansion 12.6 to 16.2 times as long as wide, 5.5 to 7.0 times as long as body diameter, and occupying 61% to 69% of total neck length; gland nuclei (obscure in most specimens) located as follows: DN = 49 ($n = 1$) and S_2N = 79–81 ($n = 3$). Nerve ring situated at 200 to 229 μm from anterior end or 19% to 21% of total neck length. Cardia rounded conoid, 15–20 × 17–18 μm; a ring-like structure is present surrounding its junction to pharyngeal base.

Female: Genital system didelphic-amphidelphic, with both branches almost equally and well developed, the anterior 698, 743 μm ($n = 2$) long or 15% of body length bearing one uterine egg inside and the posterior 545 to 630 μm long or 11% to 14% of body length (709 μm long or 14% of body length bearing one uterine egg inside). Ovaries variably sized, usually not surpassing the sphincter level, the anterior 111 to 442 μm, the posterior 115 to 435 μm long; oocytes arranged first in two or more rows, then in a single row. Oviduct 274 to 224 μm

TABLE 1. Morphometrics of *Sectonema caobangense* sp. n. Measurements in μm (except L, in mm), and in the form: average ± standard deviation (range).

		Cao Bang Natural Reserve		
Population		Holotype	Paratypes	
Character	*n*	♀	5♀♀	6♂♂
L		4.96	4.88 ± 0.67 (4.02–5.80)	3.64 ± 0.47 (3.12–4.21)
a		42	42.1 ± 6.6 (32–50)	34.1 ± 5.1 (29–42)
b		4.5	4.5, 5.4 (*n* = 2)	4.0 ± 0.3 (3.7–4.2)
c		113	111 ± 14 (98–132)	84.1 ± 5.5 (77–93)
c′		0.6	0.6 ± 0.0 (0.5–0.6)	0.7 ± 0.1 (0.6–0.8)
V		55	51.8 ± 2.8 (48–56)	54.7 ± 3.0 (51–60)
Lip region diameter		23	22.4 ± 0.9 (21–23)	22.4 ± 0.4 (22–23)
Odontostyle length at ventral side		14	13.6 ± 0.3 (13–14)	13.4 ± 0.3 (13–14)
Odontostyle length at dorsal side		16	16.4 ± 0.8 (16–17)	16.3 ± 0.4 (16–17)
Odontophore length		50	47.1 ± 1.7 (45–49)	47.0 ± 1.4 (45–49)
Guiding ring from anterior end		12	11.9 ± 1.0 (11–13)	11.1 ± 0.6 (11–12)
Neck length		1,100	1,075, 1,112 (*n* = 2)	983 ± 38 (940–1011)
Pharyngeal expansion length		745	652, 766 (*n* = 2)	649 ± 15 (632–662)
Diameter at neck base		117	107 ± 11 (91–118)	102 ± 5 (95–109)
At midbody		117	117 ± 11 (101–128)	107 ± 5 (99–113)
At anus		76	74.8 ± 4.0 (71–81)	66.2 ± 3.5 (60–71)
Prerectum length		253	202 ± 50 (150–270)	282 ± 47 (250–363)
Rectum length		51	59.5 ± 9.2 (49–66)	68.6 ± 3.4 (66–74)
Tail length		44	43.9 ± 2.3 (40–46)	43.3 ± 4.9 (36–51)
Spicule length		–	–	94.5 ± 4.5 (87–99)
Ventromedian supplements		–	–	4.7 ± 1.0 (4–6)

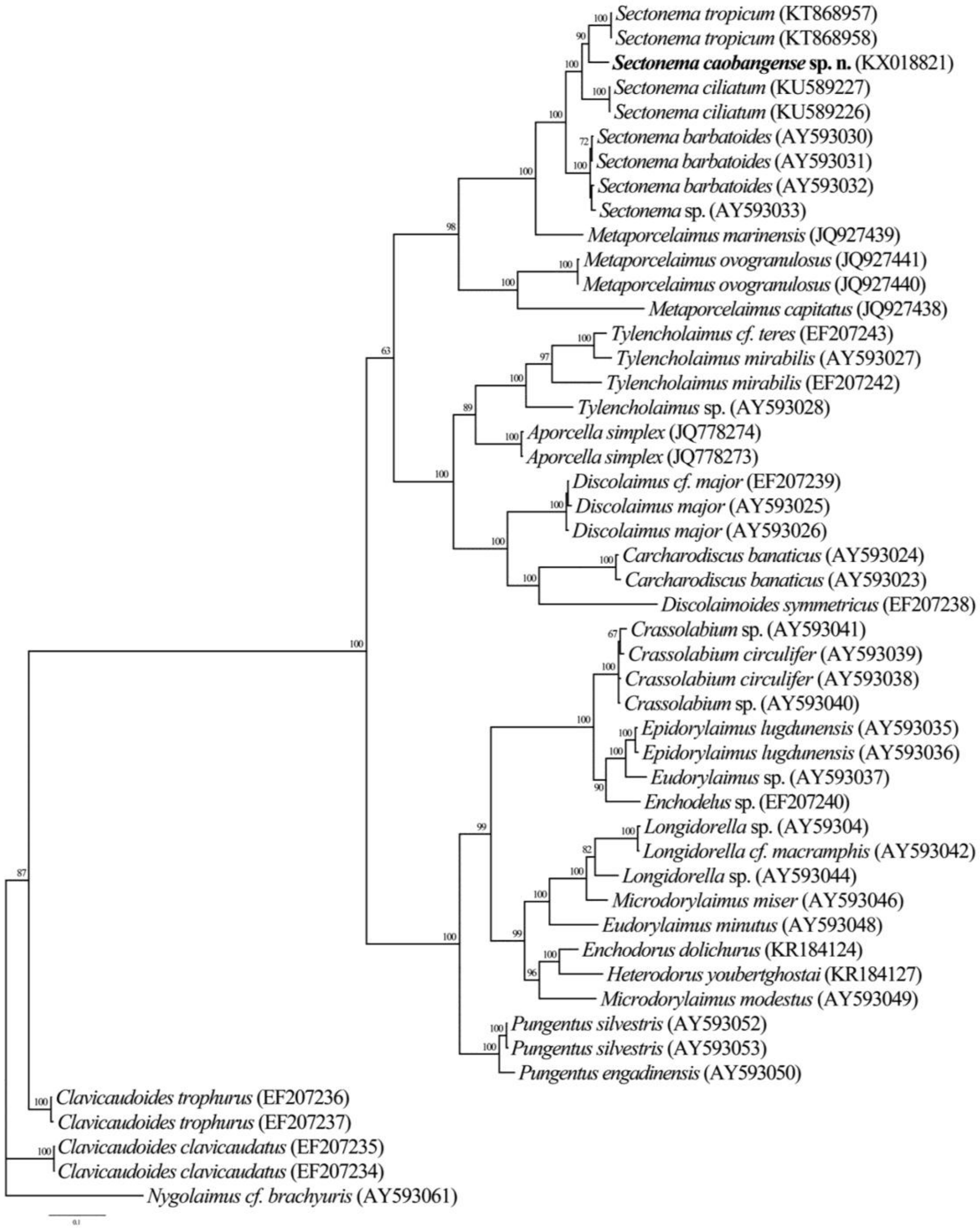

FIG. 5. Bayesian 50% majority rule consensus trees as inferred from D2-D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Posterior probabilities are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

long or 1.5 to 2.0 times the corresponding body diameter, and consisting of a slender part with prismatic cells and a well-developed *pars dilatata* bearing wide lumen that often contains sperm cells inside. Oviduct–uterus junction marked by a sphincter. Uterus a long, simple, tube-like structure 292 to 363 μm long or 2.7 to 2.9 times the corresponding body diameter (335, 341 μm long or 2.8 times the corresponding body diameter

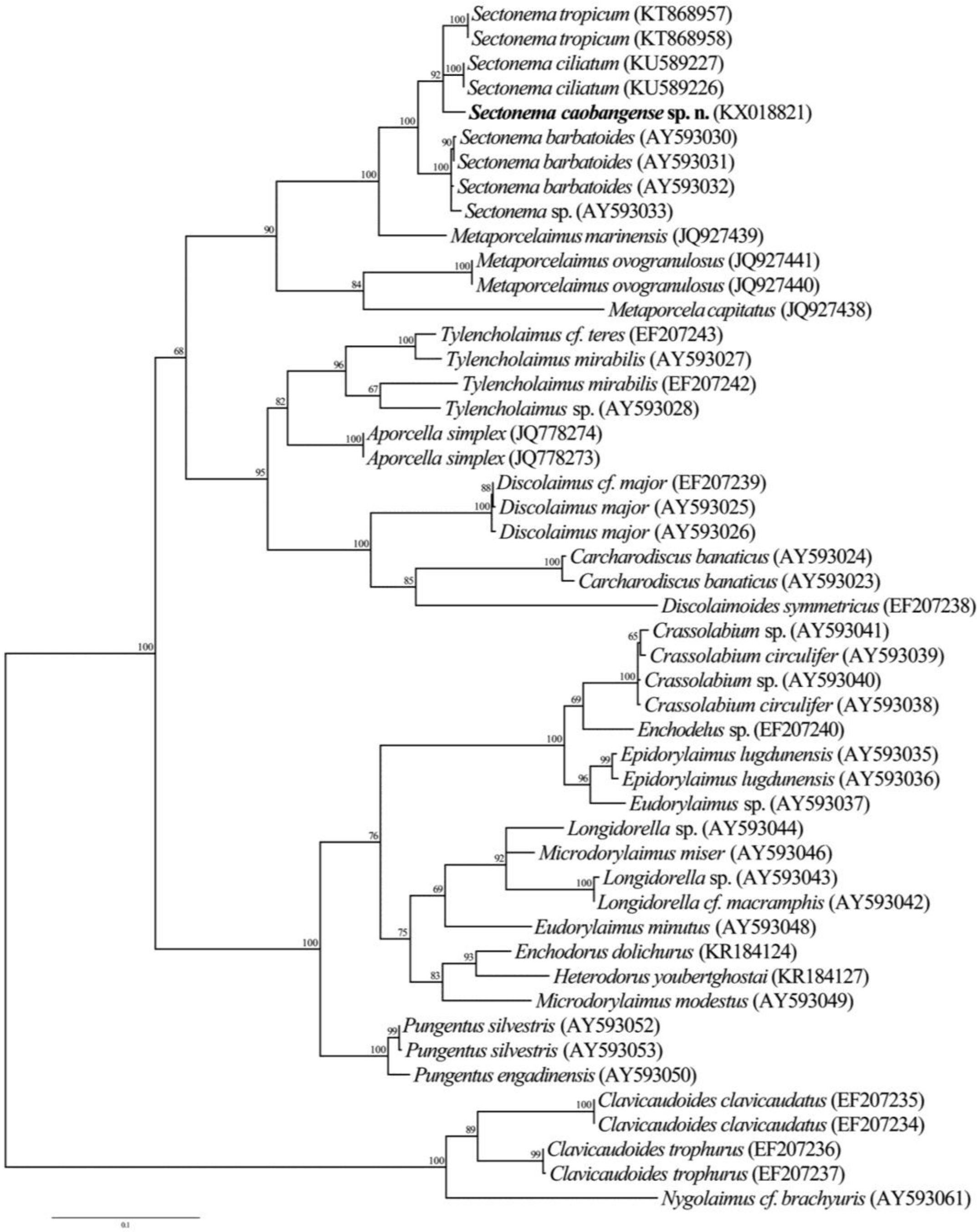

FIG. 6. Maximum likelihood tree as inferred from D2-D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Bootstrap values are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

bearing an uterine egg inside), usually containing abundant sperm cells inside. Uterine eggs ovoid, 145, 160 ($n = 2$) × 74, 84 ($n = 2$) μm, 1.7, 2.2 ($n = 2$) times as long as wide. Vagina extending inward 56 to 67 μm or one-half to three-fifths (46–57%) of body diameter: *pars proximalis* 37 to 51 × 25 to 34 μm, with somewhat sigmoid walls and surrounded by weak musculature; *pars refringens* in lateral view consisting of two triangular to drop-shaped pieces measuring 10 to 13 × 8 to 9 μm and with a combined width of 17 to 22 μm; *pars distalis* 3.5 to 7.0 μm long. Vulva a transverse slit. Prerectum 2.0 to 3.7, rectum 0.7 to 0.9 anal body diameters long. Tail short and rounded. Caudal pores two pairs, one lateral, another sub-dorsal.

Male: Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 18 to 20 μm from cloacal aperture, there is a series of four or five irregularly spaced, 13 to 56 μm apart, ventromedian supplements, the posteriormost of which lying out the range of spicules and situated at 54 to 103 μm from the ad-cloacal pair. Spicules robust and massive, especially in its posterior half, 3.6 to 4.2 times its maximum width, 1.3 to 1.6 times the body diameter at level of the cloacal aperture: dorsal contour regularly convex, ventral contour very weakly concave, with distinct hump and hollow; curvature 125 to 133°; head occupying 11% to 20% of spicule total length, its dorsal contour conspicuously curved at its anterior end and longer than the ventral one, which is short and straight; median piece 5.3 to 6.3 times longer than wide, occupying 53% to 60% of spicule maximum width, reaching the posterior tip; posterior end 7 to 9 μm wide. Lateral guiding pieces 24 to 29 μm long, 5.4 to 6.0 times longer than wide. Prerectum 3.8 to 5.5, cloaca 1.0 to 1.2 the corresponding body widths long. Tail similar to that of female. Caudal pores two pairs, one sub-lateral, another sub-dorsal.

Molecular data: One D2-D3 28S rRNA gene sequence in length of 801 bp was obtained. The evolutionary relationships of the new species with several representatives of the order Dorylaimida are presented in Figs. 5 and 6.

Diagnosis: The new species is characterized by its 3.12 to 5.80 mm long body, lip region offset by deep constriction and 21 to 23 μm broad, mural tooth 13 to 14 μm at its ventral side and 15.5 to 17.0 μm at its dorsal side, neck 940 to 1,112 μm long, pharyngeal expansion 632 to 766 μm long or occupying 61% to 69% of total neck length, uterus a simple tube-like structure 292 to 363 μm long or 2.7 to 2.9 times the corresponding body diameter, *pars refringens vaginae* well developed, V = 48 to 56, female tail short and rounded (40–46 μm, c = 98–132, c' = 0.5–0.6), male tail similar to that of female (36–51 μm, c = 77–93, c' = 0.6–0.8), spicules 87 to 99 μm long, and four or five irregularly spaced ventromedian supplements bearing hiatus.

Relationships: In the morphology of its stomatal protruding structure (mural tooth-like), the new species resembles, *S. anisonchium* Sidiqqi, 1995, *S. basilgoodeyi* Heyns, 1965 and *S. transsilvanicum* Popovici, 1978. It differs from *S. anisonchium* in its longer mural tooth (13–14 vs. 10–10.5 μm at its ventral side) having its dorsal side slightly (vs. distinctly) longer than the ventral one, rounded tail (vs. convex-conoid with broadly rounded terminus), comparatively longer male tail (c = 77–93 vs. c = 105–125), longer lateral guiding piece (24–29 vs. 13–14 μm), and lower number of ventromedian supplements (four or five vs. seven or eight). From *S. basilgoodeyi* in its longer mural tooth (13–14 vs. 12 μm at its ventral side), longer neck (940–1112 vs. 874–924 μm), more anterior vulva (V = 48–56 vs. V = 58), uterus a simple tube-like structure (vs. tripartite) and comparatively shorter (2.7–2.9 vs. about 3.4 times the corresponding body diameter), shorter spicules (87–99 vs. 110 μm), and lesser ventromedian supplements (four or five vs. eight). And from *S. transsilvanicum* in its narrower lip region (20–23 vs. 25–26 μm broad), shorter uterus (292–363 μm long or 2.7–2.9 times the corresponding body diameter vs. about 385 μm long or 3.5 times the corresponding body diameter), and shorter spicules (87–99 vs. 100–114 μm).

Molecular analyses and the derived trees (Figs. 5, 6) show that the new species forms a well-supported clade with other *Sectonema* species, especially with *S. ciliatum* Álvarez-Ortega, Nguyen, Abolafia, Vu, Bonkowski, and Peña-Santiago, 2016 and *S. tropicum* Nguyen, Abolafia, Bonkowski, Peña-Santiago and Álvarez-Ortega, 2016, two species that, however, significantly differ from *S. caobangense* sp. n. in relevant morphological features such as the nature of the stomatal protruding structure (mural tooth-like vs. a reduced odontostyle). Thus, further studies (more taxa and/or genes) should be conducted to clarify the taxonomy of the genus.

Type locality and habitat: Northern Vietnam, Cao Bang Province, Cao Bang Natural Reserve (GPS coordinates: 22° 34′07″ N and 105° 52′34″ E), in a tropical evergreen forest soil with *Dipterocarpus* sp. and *Cinnamomum* sp. as its dominant plant species.

Type material: Female holotype and four female and four male paratypes deposited in the nematode collection of the University of Jaén, Spain. One female and one male paratypes deposited in the nematode collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam.

Etymology: The specific epithet refers to geographical origin of the species.

LITERATURE CITED

Abolafia, J., and Peña-Santiago, R. 2005. Nematodes of the order Rhabditida from Andalucía Oriental: *Pseudacrobeles elongatus* (de Man, 1880) comb. n. Nematology 7:917–926.

Álvarez-Ortega, S., Nguyen, T. A. D., Abolafia, J., Vu, T. T. T., and Peña-Santiago, R. 2015. Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), with an updated taxonomy of the genus. ZooKeys 516:1–26.

Álvarez-Ortega, S., Nguyen, T. A. D., Abolafia, J., Vu, T. T. T., Bonkowski, M., and Peña-Santiago, R. 2016. Three typical species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. Nematology 18:517–536.

Álvarez-Ortega, S., Subbotin, S. A., and Peña-Santiago, R. 2013. Morphological and molecular characterization of Californian species of the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae). Nematology 15:431–439.

Baermann, G. 1917. Eine einfache methode zur auffindung von *Ankylostomum* (nematoden)-larven in erdproben. Geneeskunding Tijdschrift voor Nederlandsch-Indië 57:131–137.

Flegg, J. J. M. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. Annals of Applied Biology 60:429–437.

Heyns, J. 1965. On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimoid nematodes. Entomology Memoirs, Department of Agricultural Technical Services, Republic of South Africa 10:1–51.

Holterman, M., Rybarczyk, K., van den Elsen, S., van Megen, H., Mooyman, P., Peña-Santiago, R., Bongers, T., Bakker, J., and Helder, J. 2008. A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. Molecular Ecology Resources 8:23–34.

Huelsenbeck, J. P., and Ronquist, F. 2001. MrBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Loof, P. A. A., and Coomans, A. 1970. On the development and location of the oesophageal gland nuclei in Dorylaimina. Proceedings of the IX International Nematology Symposium, Warsaw, Poland, 1967, pp. 79–161.

Nguyen, T. A. D., Abolafia, J., Bonkowski, M., Peña-Santiago, R., and Álvarez-Ortega, S. 2016. Two atypical new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. European Journal of Taxonomy 171:1–20.

Nicholas, K. B., Nicholas, H. B., Jr., and Deerfield, D. W., II 1997. GeneDoc: Analysis and visualization of genetic variation. EMBNET NEWS 4:1–14.

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Peña-Santiago, R., Abolafia, J., and Álvarez-Ortega, S. 2014. New proposal for a detailed description of the dorylaim spicule (Nematoda: Dorylaimida). Nematology 16:1091–1095.

Popovici, P. 1978. New nematodes species (Dorylaimoidea) from Romania. Nematologica 24:404–411.

Ronquist, F., and Huelsenbeck, J. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Siddiqi, M. R. 1964. Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. Journal of Zoological Systematics and Evolutionary Research 2:174–184.

Siddiqi, M. R. 1995. Nematodes of tropical rainforests. 5. Seven new genera and forty two new species of dorylaims. Afro-Asian Journal of Nematology 5:72–109.

Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N., and Baldwin, J. G. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematology 8:455–474.

Swofford, D. L. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b 10. Sunderland, MA: Sinauer Associates.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30:2725–2729.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–4882.

Thorne, G. 1930. Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. Journal of Agricultural Research. USDA 41:445–466.

Publication 8

A Third New Species of *Aporcelinus* Andrássy, 2009 (Dorylaimida, Aporcelaimidae) from Vietnam, with the First SEM Study of a Representative of the Genus

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A Third New Species of *Aporcelinus* Andrássy, 2009 (Dorylaimida, Aporcelaimidae) from Vietnam, with the First SEM Study of a Representative of the Genus

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Abstract: A new species, the third one from Vietnam, of the genus *Aporcelinus* is described from natural areas. *Aporcelinus falcicaudatus* sp. n. is characterized by its 1.28 to 1.63 mm long body, lip region offset by weak constriction and 16 to 18 μm broad, odontostyle 18 to 21 μm at its ventral side, neck 354 to 406 μm long, uterus tripartite and 61 to 95 μm long, V = 50 to 55, tail strongly recurved dorsad and conical (23–31 μm, c = 43–58, c' = 0.7–0.9) with finely rounded tip, and male absent. Scanning electron microscope (SEM) study, the first of a representative of the genus, shows a lip region pattern significantly different from that observed in the typical aporcelaimid taxa.

Key words: aporcelaims, description, free-living nematodes, southeast Asia, taxonomy.

The genus *Aporcelinus* Andrássy, 2009 is a worldwide distributed, free-living nematode taxon, mainly dwelling soils of both natural and agricultural habitats and, more exceptionally, freshwater sediments too. It hitherto contains 18 valid species, but many of them certainly remain to be discovered. This is the second contribution in a short series devoted to studying the abundant material of this genus collected in Vietnam, the first one (Nguyen et al., 2016) focused on the description of two new species, namely *Aporcelinus paramamillatus* and *Aporcelinus paraseychellensis.*

Recently, Vinciguerra et al. (2014) raised some doubt about the taxonomy of *Aporcelinus.* It was originally classified under Aporcelaimidae Heyns, 1965, but also shows some morphological affinities with representatives of Qudsianematidae Jairajpuri, 1965. The study of a nondescribed species, the third one from Vietnam, with the first available SEM pictures of a representative of the genus, will probably provide additional evidence to clarify its taxonomical position.

MATERIALS AND METHODS

Sampling: Soil samples were collected in a natural area on Cat Ba Island of northern Vietnam and temporally stored in plastic bags to transport them to the laboratory. Nematode extraction was made following the methods by Baermann (1917) and Flegg (1967). Then, the worms were relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerol according with Siddiqi's (1964) technique, and mounted on permanent glass slides for their handling and observation under light microscope.

Light microscopy: Measurements, line drawings, and pictures were taken using a Nikon Eclipse 80i light microscope provided with differential interference contrast optics, a Nikon Digital Sight DS-U1 camera and a drawing tube. Morphometrics include Demanian ratios as well as other relevant measurements, and are presented in Table 1. Raw photographs were edited using Adobe Photoshop CS.

Scanning electron microscopy: After their examination and identification, one specimen in good condition was selected to its observation under SEM following the protocol by Abolafia (2015). The nematode was hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope.

RESULTS

Aporcelinus falcicaudatus[*] sp. n.

(Figs. 1-3; Table 1)

Material examined: Nine females, in variable state of preservation.

Description

Female: Moderately slender (a = 23–28) nematodes of medium size, 1.28 to 1.63 mm long. Habitus visibly curved ventrad on fixation, C- or G-shaped. Cuticle two-layered, 3 to 4 μm thick at anterior region and midbody and 6 to 7 μm on tail; outer layer thin and bearing fine but conspicuous transverse striation throughout the body, specially visible under SEM; inner layer thicker and more refractive than the outer layer. Lateral chord 4 to 9 μm broad or 5% to 13% of midbody diameter. Body pores often obscure, but two cervical pores are visible at both dorsal and ventral sides at level of odontostyle plus odontophore. Lip region angular, offset by a shallow constriction, 2.7 to 3.0 times as wide

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[*] Urn:lsid:zoobank.org:act:91CD2CA7-4809-4D27-89E0-F3C61F010EB5.

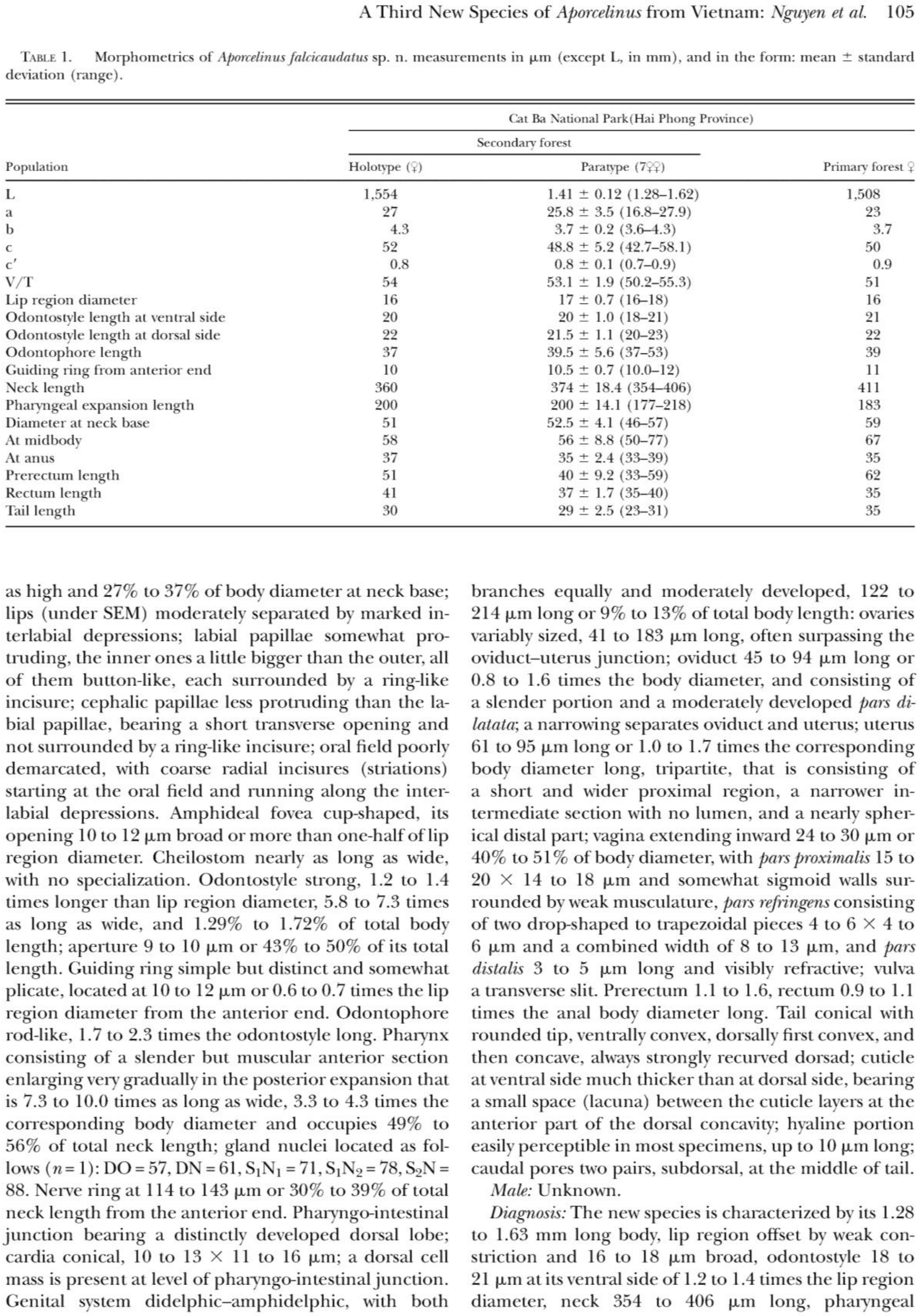

TABLE 1. Morphometrics of *Aporcelinus falcicaudatus* sp. n. measurements in μm (except L, in mm), and in the form: mean ± standard deviation (range).

	Cat Ba National Park(Hai Phong Province)		
	Secondary forest		
Population	Holotype (♀)	Paratype (7♀♀)	Primary forest ♀
L	1,554	1.41 ± 0.12 (1.28–1.62)	1,508
a	27	25.8 ± 3.5 (16.8–27.9)	23
b	4.3	3.7 ± 0.2 (3.6–4.3)	3.7
c	52	48.8 ± 5.2 (42.7–58.1)	50
c′	0.8	0.8 ± 0.1 (0.7–0.9)	0.9
V/T	54	53.1 ± 1.9 (50.2–55.3)	51
Lip region diameter	16	17 ± 0.7 (16–18)	16
Odontostyle length at ventral side	20	20 ± 1.0 (18–21)	21
Odontostyle length at dorsal side	22	21.5 ± 1.1 (20–23)	22
Odontophore length	37	39.5 ± 5.6 (37–53)	39
Guiding ring from anterior end	10	10.5 ± 0.7 (10.0–12)	11
Neck length	360	374 ± 18.4 (354–406)	411
Pharyngeal expansion length	200	200 ± 14.1 (177–218)	183
Diameter at neck base	51	52.5 ± 4.1 (46–57)	59
At midbody	58	56 ± 8.8 (50–77)	67
At anus	37	35 ± 2.4 (33–39)	35
Prerectum length	51	40 ± 9.2 (33–59)	62
Rectum length	41	37 ± 1.7 (35–40)	35
Tail length	30	29 ± 2.5 (23–31)	35

as high and 27% to 37% of body diameter at neck base; lips (under SEM) moderately separated by marked interlabial depressions; labial papillae somewhat protruding, the inner ones a little bigger than the outer, all of them button-like, each surrounded by a ring-like incisure; cephalic papillae less protruding than the labial papillae, bearing a short transverse opening and not surrounded by a ring-like incisure; oral field poorly demarcated, with coarse radial incisures (striations) starting at the oral field and running along the interlabial depressions. Amphideal fovea cup-shaped, its opening 10 to 12 μm broad or more than one-half of lip region diameter. Cheilostom nearly as long as wide, with no specialization. Odontostyle strong, 1.2 to 1.4 times longer than lip region diameter, 5.8 to 7.3 times as long as wide, and 1.29% to 1.72% of total body length; aperture 9 to 10 μm or 43% to 50% of its total length. Guiding ring simple but distinct and somewhat plicate, located at 10 to 12 μm or 0.6 to 0.7 times the lip region diameter from the anterior end. Odontophore rod-like, 1.7 to 2.3 times the odontostyle long. Pharynx consisting of a slender but muscular anterior section enlarging very gradually in the posterior expansion that is 7.3 to 10.0 times as long as wide, 3.3 to 4.3 times the corresponding body diameter and occupies 49% to 56% of total neck length; gland nuclei located as follows ($n = 1$): DO = 57, DN = 61, S_1N_1 = 71, S_1N_2 = 78, S_2N = 88. Nerve ring at 114 to 143 μm or 30% to 39% of total neck length from the anterior end. Pharyngo-intestinal junction bearing a distinctly developed dorsal lobe; cardia conical, 10 to 13 × 11 to 16 μm; a dorsal cell mass is present at level of pharyngo-intestinal junction. Genital system didelphic–amphidelphic, with both branches equally and moderately developed, 122 to 214 μm long or 9% to 13% of total body length: ovaries variably sized, 41 to 183 μm long, often surpassing the oviduct–uterus junction; oviduct 45 to 94 μm long or 0.8 to 1.6 times the body diameter, and consisting of a slender portion and a moderately developed *pars dilatata*; a narrowing separates oviduct and uterus; uterus 61 to 95 μm long or 1.0 to 1.7 times the corresponding body diameter long, tripartite, that is consisting of a short and wider proximal region, a narrower intermediate section with no lumen, and a nearly spherical distal part; vagina extending inward 24 to 30 μm or 40% to 51% of body diameter, with *pars proximalis* 15 to 20 × 14 to 18 μm and somewhat sigmoid walls surrounded by weak musculature, *pars refringens* consisting of two drop-shaped to trapezoidal pieces 4 to 6 × 4 to 6 μm and a combined width of 8 to 13 μm, and *pars distalis* 3 to 5 μm long and visibly refractive; vulva a transverse slit. Prerectum 1.1 to 1.6, rectum 0.9 to 1.1 times the anal body diameter long. Tail conical with rounded tip, ventrally convex, dorsally first convex, and then concave, always strongly recurved dorsad; cuticle at ventral side much thicker than at dorsal side, bearing a small space (lacuna) between the cuticle layers at the anterior part of the dorsal concavity; hyaline portion easily perceptible in most specimens, up to 10 μm long; caudal pores two pairs, subdorsal, at the middle of tail.

Male: Unknown.

Diagnosis: The new species is characterized by its 1.28 to 1.63 mm long body, lip region offset by weak constriction and 16 to 18 μm broad, odontostyle 18 to 21 μm at its ventral side of 1.2 to 1.4 times the lip region diameter, neck 354 to 406 μm long, pharyngeal

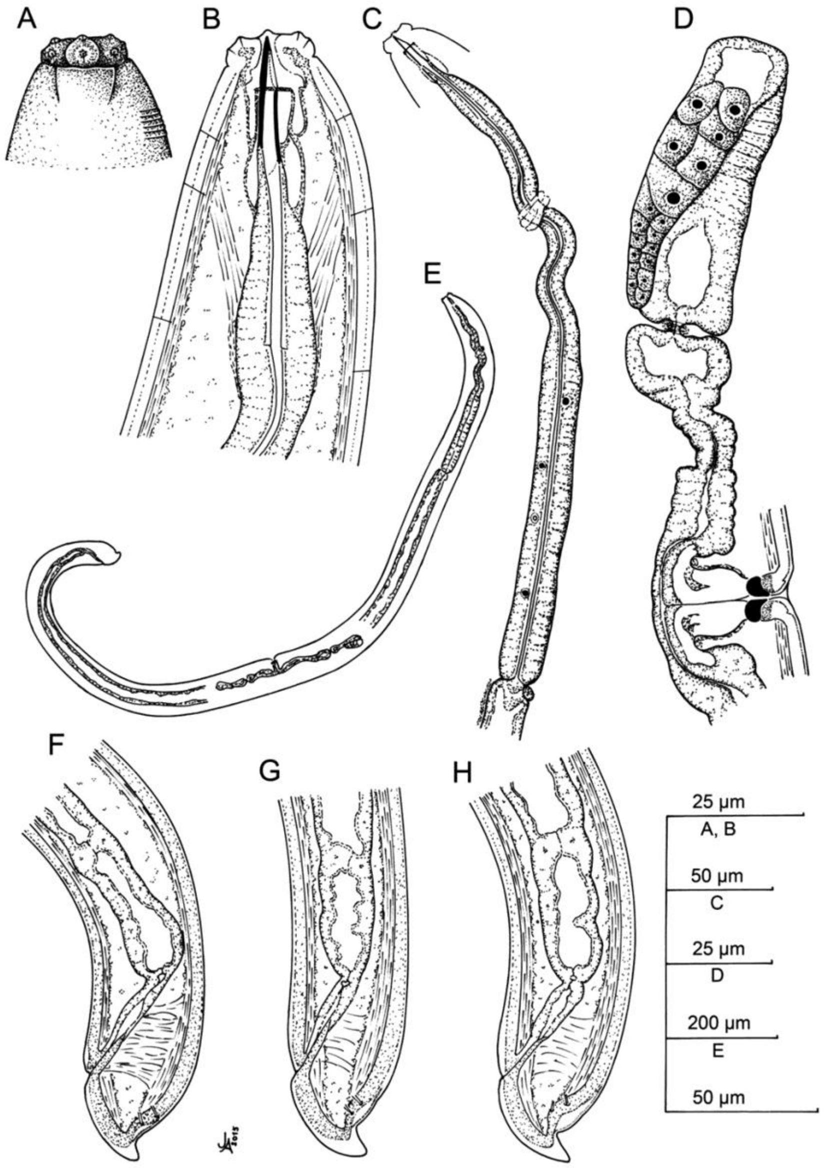

FIG. 1. *Aporcelinus falcicaudatus* sp. n. (Female). A. Lip region in surface, lateral view. B. Anterior region in median, lateral view. C. Neck region. D. Anterior genital branch. E. Entire. F–H. Posterior body region.

expansion 177 to 218 µm long or 49% to 56% of total neck length, a dorsal cell mass present at the pharyngo-intestinal junction, uterus tripartite and 61 to 95 µm long or 1.0 to 1.7 times the corresponding body diameter long, $V = 50$ to 55, tail (23–31 µm, $c = 43–58$, $c' = 0.7–0.9$) strongly recurved dorsad and conical with finely rounded tip, and male absent.

Relationships: The new species is easily recognizable by the morphology of its caudal region (Fig. 2H–N): strongly recurved dorsad, cuticle at ventral side much thicker than at dorsal side, the presence of a hyaline small space (lacuna) between the two cuticle layers at level of the dorsal concavity, and a conspicuous terminal hyaline portion.

In its general morphometrics (medium-sized body, lip region up to 18 µm broad, odontostyle ca. 20 µm long) and caudal region with a distinct dorsal concavity, this species resembles *A. irritans* (Cobb in Thorne and Swanger, 1936) Andrássy, 2009, *A. jiaonanensis* (Zhao and Zhao, 2010) Álvarez-Ortega and Peña-Santiago, 2013, and *A. seychellensis* (Andrássy, 2011) Andrássy, 2009, but it differs

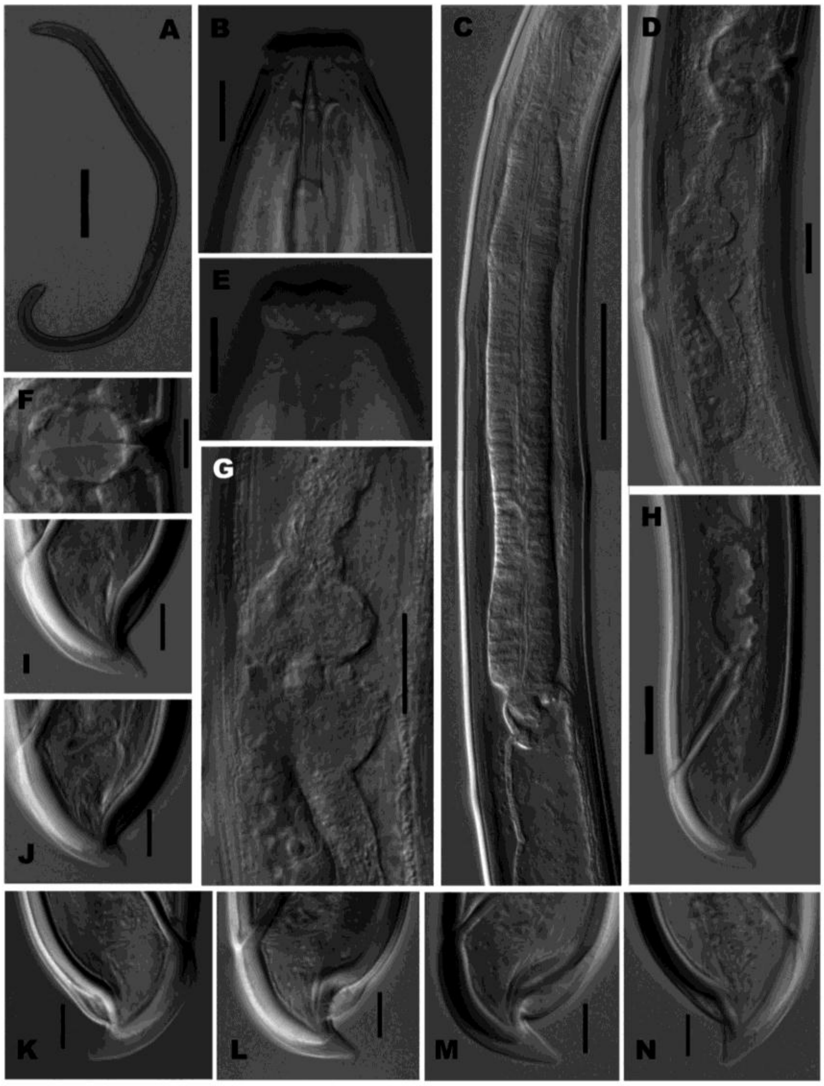

FIG. 2. *Aporcelinus falcicaudatus* sp. n. (Female, light microscopy). A. Entire. B. Anterior region in median view. C. Pharyngeal expansion and pharyngo-intestinal junction. D. Posterior genital branch. E. Lip region in lateral, surface view. F. Vagina. G. Oviduct–uterus junction. H. Posterior body region. I–N. Caudal region. (Scale bars: A = 200 µm; B, E, F, I–N = 10 µm; C = 50 µm; D, G, H = 20 µm.)

from these in its strongly (vs. not distinctly) recurved dorsad caudal region. Besides, it can be distinguished from *A. irritans*, a poorly known species, in its broader lip region (16–18 vs. 14–15 µm) and much shorter caudal region (23–31 µm, *c* = 47–58, *c′* = 0.7–0.9 vs. 39–42 µm, *c* = 36, *c′* = 1.3–1.4); from *A. jiaonanensis* in its much shorter caudal region (23–31 µm, *c* = 47–58, *c′* = 0.7–0.9 vs. 38–46 µm, *c* = 32–40, *c′* = 1.1–1.4); and from *A. seychellensis* in its shorter caudal region (23–31 µm, *c* = 47–58 vs. 33–43 µm, *c* = 25–42) with rounded (vs. very acute) tip.

Type locality and habitat

Holotype: Northern Vietnam, Cat Ba National Park, Hai Phong Province (GPS coordinates: 20° 47′45″ N and 107° 00′40″E, elevation 170 m), in soil of a secondary forest with *Cinnamomum* sp., *Machilus* sp., and *Dimocarpus* sp. as dominant plants. Deposited on slide Cat Ba 13.02 in the nematode collection of the University of Jaén, Spain.

Paratypes: Same locality and habitat that holotype. Seven paratypes deposited on slides Cat Ba 13.01 and 13.02 in the nematode collection of the University of Jaén, Spain. One paratype on slide Cat Ba 22.02 in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Etymology: The specific epithet is a compound Latin term meaning "sickle-shaped tail."

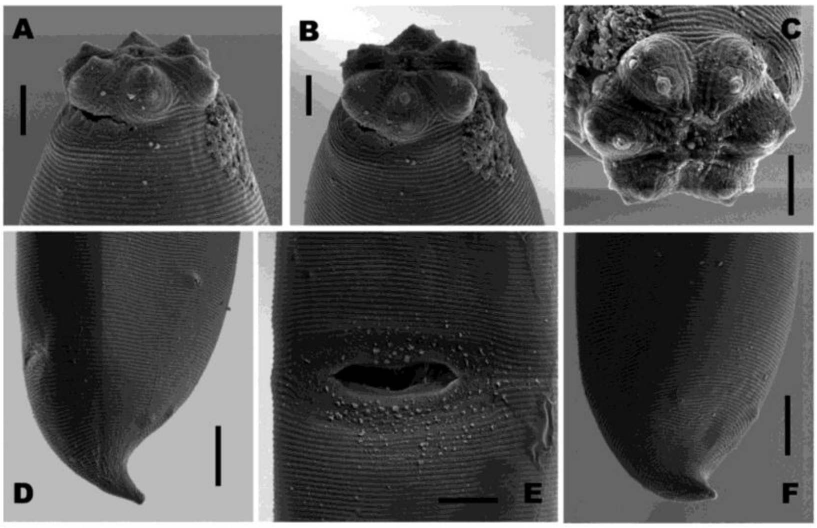

FIG. 3. *Aporcelinus falcicaudatus* sp. n. (Female, scanning electron microscope). A, B. Anterior region in sublateral view. Entire. C. Lip region in face view. D. Caudal region in lateral view. E. Vulva in ventral view. F. Caudal region in subdorsal view. (Scale bars: A–C = 5 µm; D–F = 10 µm.)

DISCUSSION

As mentioned in the introductory section, Andrássy (2009) originally classified *Aporcelinus* under Aporcelaimidae, an action that has been recently questioned by Vinciguerra et al. (2014). The first SEM study of a representative of *Aporcelinus* is herein provided. Its results show that the lip region pattern significantly differs from that observed in the typical aporcelaims, which is characterized by having a biradial symmetry, oral aperture a large, dorsoventral, hexagonal slit, and a distinctly demarcated oral field (for instance, see Álvarez-Ortega et al. [2012] for *Aporcelaimellus communis*; Nguyen et al. [2016] for *Sectonema* spp.). Then, Vinciguerra et al.'s opinion is herein supported. Nevertheless, further data should be obtained, especially molecular analyses, to solve definitively the matter.

LITERATURE CITED

Abolafia, J. 2015. A low-cost technique to manufacture a container to process meiofauna for scanning electron microscopy. Microscopy Research and Technique 78:771–776.

Álvarez-Ortega, S., Abolafia, J., Liébanas, G., and Peña-Santiago, R. 2012. Studies on the genus *Aporcelaimellus* Heyns, 1965 (Nematoda, Dorylaimida, Aporcelaimidae). Four new species with complex uterus from Southeastern Iberian Peninsula. Zootaxa 3551:1–24.

Álvarez-Ortega, S., and Peña-Santiago, R. 2013. Taxonomy of the genus *Aporcelaimellus* Heyns, 1965 (Nematoda, Dorylaimida, Aporcelaimidae). Zootaxa 3669:243–260.

Andrássy, I. 2009. *Aporcelinus*, a new genus of aporcelaimoid nematodes (Dorylaimida), and its species. International Journal of Nematology 19:121–136.

Andrássy, I. 2011. Phylum Nematoda. Pp. 15–57 *in* J. Gerlach, ed. Crustacea, Platyhelminthes, Nematoda, Annelida and Tardigrada of the Seychelles Islands, Manchester: Siri Scientific Press.

Baermann, G. 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneeskunding Tijdschrift voor Nederlandsch–Indië 57:131–137.

Cobb, N. A. 1932. The English word "nema". Journal of the American Medical Association 98:75.

Flegg, J. J. M. 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. Annals of Applied Biology 60:429–437.

Heyns, J. 1965. On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimoid nematodes. Entomology Memoirs, Department of Agricultural Technical Services. Republic of South Africa 10:1–51.

Jairajpuri, M. S. 1965. *Qudsianema amabilis* n. gen., n. sp. (Nematoda: Dorylaimoidea) from India. Proceedings of the Helminthological Society of Washington 32:7273.

Nguyen, T. A. D., Abolafia, J., Bonkowski, M., and Peña-Santiago, R. 2016. Two new species of the genus *Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. Zootaxa 4103:550.

Nguyen, T. A. D., Abolafia, J., Bonkowski, M., Peña-Santiago, R., and Álvarez-Ortega, S. 2016. Two atypical new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. European Journal of Taxonomy 171:1–20.

Siddiqi, M. R. 1964. Studies on *Discolaimus* spp. (Nematoda: Dorylaimidae) from India. Zeitschrift für Zoologische Systematik und Evolutionsforschung 2:174–184.

Thorne, G., and Swanger, H. H. 1936. A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n. g., *Dorylaimoides* n.g. and *Pungentus* n.g. Capita Zoologica 6:1–223.

Vinciguerra, M. T., Orselli, L., and Clausi, M. 2014. One new and two known species of *Aporcelinus* Andrássy, 2009 and a new species of *Coomansinema* Ahmad & Jairajpuri, 1989 (Nematoda: Dorylaimida). Nematology 16:303–322.

Zhao, C., and Zhao, H. 2010. [A new species of the genus *Aporcelaimellus* Heyns, 1965 from Shandong, China (Nematoda, Dorylaimida, Aporcelaimidae)]. Acta Zootaxonomica Sinica 35:876–879.(In Chinese.).

Publication 9

Shifts in nematode community composition and function due to land-use intensification in tropical soils of Vietnam

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In preparation

Shifts in nematode community composition and function due to land-use intensification in tropical soils of Vietnam

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Abstract

Increasing land-use intensity has likely strong impacts on soil biodiversity and fertility in the tropics. Nematodes are known to respond rapidly to soil disturbance and changing resources. Therefore the functional composition of the nematode community offers a reliable measure for the biological assessment of the quality and functioning of tropical soils. In Northern Vietnam, we investigated the nematode community structure with the land use intensity in soils of undisturbed primary forest, soils under secondary forests, soils affected by slash and burn agriculture, and intensively managed agricultural land with rice and corn crops in Pia Oac Natural Reserve, Cao Bang Provinces. First, we identified the nematodes soil fauna until the species level (if possible) to get estimate on the numbers and diversity of known and undescribed taxa. In total, more than 4800 specimens were identified belonging to 77 genera. Then, based on the nematode trophic group, we determine how the land used type affect to the nematode community by using different ecological indices and food web analysis. Remarkably, the diversity index showed the higher diversity of nematode community in intensive agriculture land than in the forest while the total nematodes abundance was not significant different. In this context, the Maturity Index, Channel Index and Plant Parasitic Index clearly indicated the different nematodes community composition in intensive agriculture with others. Furthermore, we proposed the network structure for nematode community which can be explain the shift in land use. Finally, the abiotic factor such as the C/N ratio did not affect the nematodes community.

Keywords: Land use type, nematodes community composition, tropical soil, Vietnam

1. Introduction

Deforestation and conversion of forest land for agriculture has increased dramatically in Southeast Asia since the 1980’s (Hansen and DeFries, 2004). The current deforestation rates recorded in this region are the highest worldwide (Achard, 2002; Shearman et al., 2012). In Vietnam, data from the Forest Resources Assessment (FAO, 1993) showed the highest rates of deforestation during the last decade and illegal logging destroyed large forest regions during the 1990s.

Nematodes are important components of soil microfauna. Millions of nematodes per square meter can be found in any kind of natural (but also agro-) system, and there are several dozens of species in a handful of soil (Bongers and Ferris, 1999). The free-living soil nematodes play an important role in the decomposition pathways of soil food webs (de Ruiter et al., 1998). Moreover, these nematodes interact, directly or indirectly, with a variety of soil organisms, from bacteria (Traunspurger et al., 1997; de Mesel et al., 2004; Moens et al., 2005), fungi and earthworms to arthropods and molluscs. Especially, predators and omnivore nematodes are sensitive to any kind of changes (Korthals et al., 1996; Tenuta and Ferris, 2004). These biotic interactions as well as their relationships with abiotic factor of their environment favor their potential use to characterize the state of soil microfauna and the general condition of their soil habitat.

Nematode diversity studies have historically focused on temperate regions and only ten of 134 published ecosystem surveys represented tropical forests (Boag and Yeates, 1998). Almost 20 years later, soil nematode assemblages of tropical forests are still largely unexplored. Despite earlier studies suggested a peak of nematode diversity in temperate regions (Boag and Yeates, 1998), recent studies have shown that tropical nematode communities can be extremely species rich. Bloemer at al., (1997) investigating from 24 sites of the tropical forest in Mbalmayo, Cameroon found 431 species belonging to 194 genera and from individual soil cores contained a minimum of 61 species and maximum of an average 89 species. Very recently, Porazinska et al., (2010, 2012) found for tropical forest in South America that total species richness was 300% higher in tropical than in temperate forests and more than 90% of the identified species belonged to new forms. The low diversities reported earlier could be artefacts due to an inappropriate soil sampling strategy for the tropics (Lawton et al., 1996; Powers et al., 2009). Although nematode diversity is know from many study sites in the world, little attention has been paid to the question of how local nematode diversity depends on regional species richness and how land use intensity management affect to natural species richness. Because tropical rainforests on different continents are characterized by vastly different abiotic and biotic conditions and evolved rather independently, we are not only lacking basic knowledge on the species inventory, but more so on the regional drivers of diversity and community assembly in these tropical regions (Corlett and Primack, 2006; Kim and Byrne, 2006; Grainger, 2010).

Different nematode genera and species are specialized to use very different food resources. Based on the natural structure of their feeding apparatus, nematodes are placed in five distinct functional groups (Yeates, 1993; Yeates and Bongers, 1999): bacterivores, fungivores, herbivores, omnivores and predators (Yeates et al., 1993). Several ecological indices base on trophic group of nematodes such as: Maturity Index (MI), MI2-5, ΣMI2-5, PPI, Basal index, Enrichment index, Structure index and Channel Index can be used to measure the shift of the nematode community in functioning and services ecosystems. Major aims of this study were: 1) To investigate the taxonomic diversity of free-living nematodes species in tropical forest along land use gradients. 2) To determine how increasing land-use intensity effects their functional roles in the soil food web (i.e. functional group level).

2. Materials and Methods

2.1. Study area

The study was conducted in Pia Oac Nature Reserve ($22^{0}32^{'}$ – $22^{0}40^{'}$N, $105^{0}49^{'}$ – $105^{0}57^{'}$E), Cao Bang Province in the north-east of Vietnam. The climate conditions correspond to subtropical with a mean annual temperature of 25^{o}C and the lowest temperatures in January and the highest in July. The mean of an annual precipitation is approximately 1400 mm and the relative ambient humidity of 80% (Sterling et al., 2006). Tropical forests in Pia Oac are situated on limestone karst mountains at elevations of 1000 – 1931 m. Study sites were selected in these areas of Pia Oac Nature Reserve and each comprised primary forest, secondary forest, former slash and burn agicultural land and intensively managed agriculture land with rice and maize cultivation.

2.2. Soil sampling

At each study site, soil nematode samples have been taken in May, 2013 (Fig. 1) in four different land use types: i) largely undisturbed primary forest, ii) disturbed secondary forest, iii) cassava fields characteristic of slash and burn, and iv)agriculture intensively managed agricultural fields (maize, rice). In each land-use type, six bulk soil samples were taken. Each bulk soil sample was pooled by mixing 5 soil cores in a crosswise grid design in a 10 m^2 plot. After removal of the litter layer, the soil samples were collected from 0-10 cm depth with a 3.5 cm diameter soil corer. In total, 24 composite soil samples were collected in each site as independent replicates. Each soil was mixed and passed through a sieve with 2mm mesh size to remove small stones and roots. A part of the soil was used for measurements of chemical properties (soil moisture, total C, N) and the other part was used for nematode extraction.

2.3 Nematode community analysis

Nematodes were extracted from 100 fresh g of soil samples by extraction technique (Bearmann, 1917) and Flegg's (1967) sieving method. The extracted nematodes were relaxed, killed by hot water (70^{o}C), and fixed in 4% formaldehyde. Total nematode numbers were counted under stereomicroscope (x50 magnification) and 200 individuals were picked out randomly for indentification. The fixed individuals were gradually transfered to anhydrous glycerol following De Grisse's (1969) technique, and mounted on permanent glass slides. The nematodes were identified to genus level by using the keys from Andrássy, 2009, Ahmad & Jairajpuri, 2010.

Functional groups of nematodes were assigned according to Yeates et al., 1993: 1) bacterivore nematode (Bax), 2) fungivore nematode (Fux), 3) herbivore nematode (Plant parasitic nematode) (PPI), 4) omnivore nematode (Omx), and 5) Predator (Cax). Colonizer-persistor c-p values were assigned to taxonomic group according to (Bongers, 1990).

The data were presented according to the following parametters: total abundance, trophic group. Taxonomic diversity was calculated using the total number of genera (S) in a sample, Shannon-Wiener diversity index (H'), Pielou's eveness (J').

Maturity index (MI) and Plant Parasitic Index (PPI) was calculated according to Bongers (1990). MI (or PPI) = $\sum_{i=1}^{n} v(i) * f(i)$, where v(i) is the c-p (coloniser-persister) value of free-living (or plant-feeding) nematode taxon i; f(i) is the frequency of free-living (or plant-feeding) nematode taxon *i* in a sample. Low values MI and PPI indicating disturbance or a succession stage with dominance of r-strategists. The MI2-5 index is similar to the MI except that MI2-5 excludes the c-p1 enrichment opportunistic species.

Ferris et al. (2001) proposed the Enrichment Index (EI), Struture Index (SI) and Channel Index (CI) to evaluate food web characteristic. These indices are calculated from the weighted abundance of nematode guilds. Bacterivores (Bax), fungivores (Fux), predators (Prx), and omnivores (Omx) are considered as nematode guilds ranging along the c–p scale from x = 1 to x = 5. The nematode fauna is comprised of basal, enrichment and structural components (b,e,s):

b=(Ba2+Fu2)*W_2, where W.= 0.8,

e=(Ba1*W_1)+(Fu2*W_2), where W_1 = 3.2 and W_2.= 0.8

$s=(Ba_n*W_n+Ca_n*W_n+Fu_n*W_n+Om*W_n)$ where $n=3\text{-}5$, $W_3.= 1.8$, $W_4.= 3.2$, $W_5= 5.0$

The Enrichment (EI), Structure (SI), Basal (BI), and Channel (CI) indices were calculated from the weighted faunal components (Ferris et al., 2001; Berkelmans et al., 2003):

$EI =100*e/(e+b)$

$SI = 100*s/(s+b)$

$BI = 100*b/(e+s+b)$

$CI = 100\ Fu2*W_2/(Ba1*W_1+Fu2*W_2)$.

2.4. Soil analysis: Carbon and nitrogen content

The C and N content and the carbon-nitrogen ratio (C/N) of the soil samples were measured from dried and mineral soil with a NC Analyzer (Flash 2000, Thermo Scientific), USA.

2.5. Statistical analysis

Statistically analyses were performed in R, version 3.2.2. The community data on genus level (and on the functional group level) based on Bray-Curtis similarity was analysed to assess the differences between the location and land-use intensity (LUI). Non-metric multidimensional scaling (NMDS) was used to visualize the results. Analysis of Variance (ANOVA) and subsequent Tukey Tests were used to analyse the effects of regions and LUI on nematode taxa and trophic groups. Differences at the $P < 0.05$ level were considered to be statistically significant. Additionally a Permutation Multivariate Analysis of Variance (PERMANOVA), Discriminant Function Analyses (DFAs) and Pearson product-moment correlation (Pearson-correlation) were applied for the data.

Soil nematodes indices such as MI, PPI, CI and Enrichment index calculated NINJA website (Nematode INdicator Joint Analysis: https://sieriebriennikov.shinyapps.io/ninja/)

Results

3.1. Nematode diversity

A total of 77 nematode genera were found in our study (Appendix. 1). Herbivores and bacterivores were predominant in the agroecosystem while fungivores, omonivores and predators dominated in the natural forests. Three omnivorous nematodes: *Eudorylaimus*, *Mesodorylaimus* and *Oriverutus* were the most abundant genera in primary forest and secondary forest, and together represented 47% and 44% of total nematode abundance. In primary forest, the nematode genus *Alaimus* was dominant (16%) among the bacterivore. The fungivorous genera: *Aphelenchoides*, *Aphelenchus* and *Filenchus* dominanted in the slash and burn and intensive agriculture. *Helicotylenchus* was the most dominant of herbivores nematode in intensive agriculture. The genera: *Rotylenchus, Psilenchus, Filenchus, Acrobeloides, Eucephalobus* and *Ironus* were only found in soil of slash and burn and intensive agriculture (Table 1).

The total abundance of nematodes ranged between 380 and 2400 ind./100 g soil was not different between land use types (F=1.97, P = 0.15). Nematode abundance was highest in the secondary forest (2400ind./100 g) followed by primary forest (1800ind./100 g) while it was lowest in the intenisive agriculture (380ind./100 g). The abundance of plant parasitic nematodes was highest in intensive agriculture. The abudance of omnivores and predators nematode showed a general decline from the forest, reflecting dominance by plant parasitic nematodes in intensive agriculture.

In our study, a phylogenetic tree was constructed based on 67 sequences (Fig. 1) of the large 28S subunit (LSU) of the ribosome (rDNA) in nematodes from Cao Bang associated with mineral soil. However, only 5 sequences obtained from Genbank could be assigned to the sequenced nematodes with a similarity higher than 95%. This clearly demonstrated that the molecular diversity of dorylaimids in Vietnam is completely undiscovered

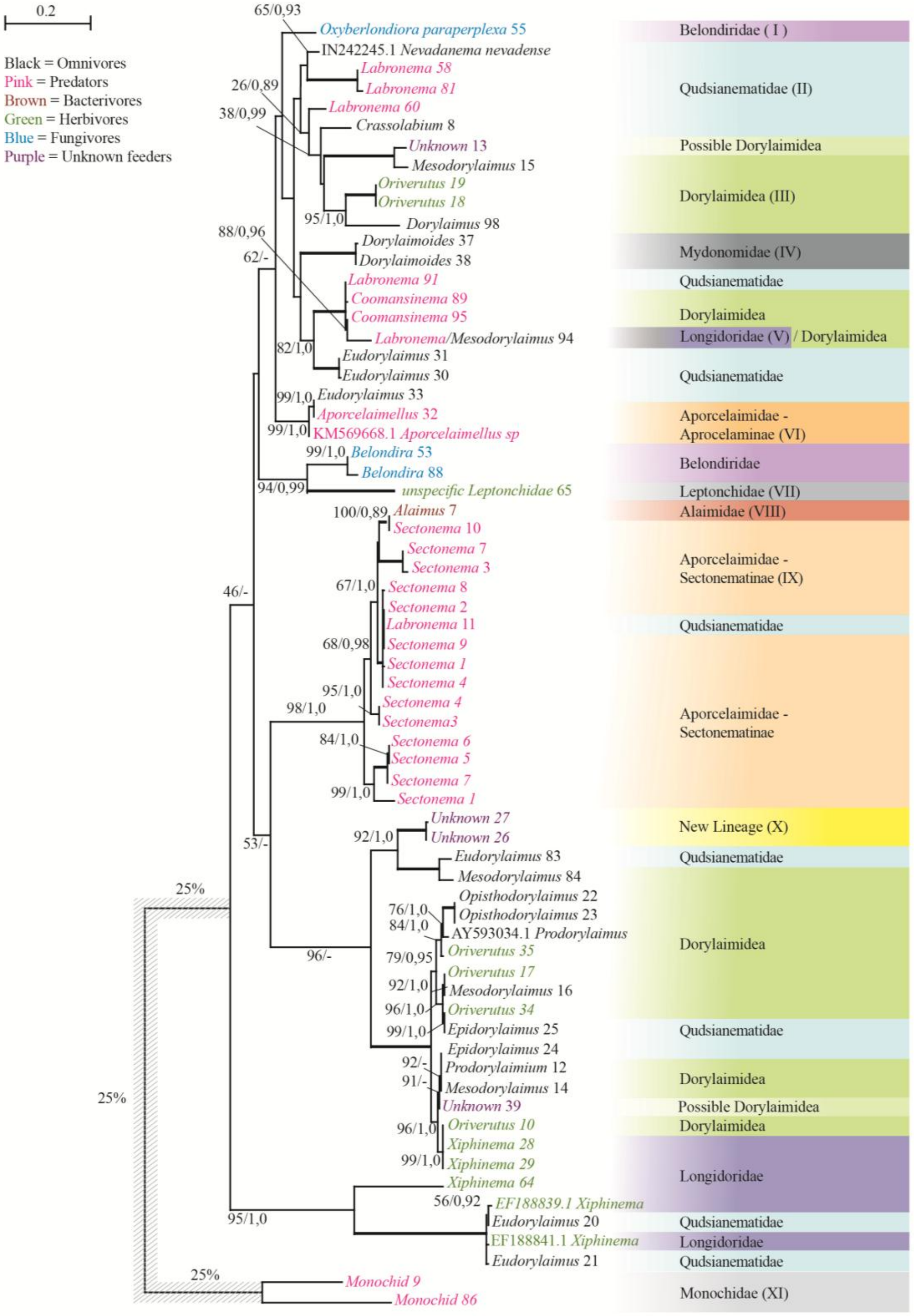

Figure 1**.** Phylogeny tree of 67 sequences (28S rDNA) based on pair wise. Different colours of taxa indicate different feeding types of nematodes according to Yeates *et al*. (1993). Black = Omnivores; Pink = Predators; Brown = Bacterivores; Green = Herbivores; Blue = Fungivores; Purple = Unknown feeder. From the 67 specimens, 62 individuals with were determined according to morphology, giving 41 putative species of 18 genera belonging to 9 families of the order Dorylaimida, but 5 individuals could not be assigned to known taxa.

Table 1: Mean relative density of common and rare nematode genera (ind./100g soil) in different land use intensity. Values are means and standard deviation, (n=6). F and P values of the effect of LUI are presented. (Significant of effect: P< 0.5: ‘ ’; P < 0.1, ‘*’; p< 0.05 ‘**’; p< 0.001 ‘***’).

Genera	Primary forest (n=6)		Secondary forest (n=6)		Slash and burn (n=6)		Intensive Agriculture (n=6)		F	P
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev		
Bacterivores										
Acrobeloides	0	0	0	0	0	0	14.8	15.5	5.5	0.06 **
Alaimus	143	86.2	67.8	79.2	61.7	46.2	54.2	79.1	1.9	0.16
Daptonema	15.4	17.8	7.0	10.1	5.0	8.9	0.8	2.0	1.8	0.17
Eucephalobus	0	0	0	0	1.0	2.4	95.8	98.3	5.7	0.05**
Fungivores										
Aphelenchoides	6.1	12.1	25.1	22.2	9.7	14.0	102	74.1	7.7	0.001**
Filenchus	0	0	0	0	4.3	4.7	54.0	30	18.1	<01***
Herbivores										
Belondira	2.5	6.1	3.7	6.1	3.7	6.7	18.5	25.6	1.8	0.17
Helichotylenchus	0	0	27	57	31	53	10	14	0.8	0.5
Psilenchus	0	0	0	0	2.5	3.9	18.8	22.7	3.7	0.02 *
Rotylenchus	0	0	1.3	3.2	0	0	11.3	13.3	3.8	0.02 *
Tylenchus	1.2	2.9	12.2	10.7	9.3	14.8	12.3	23.2	1.8	0.17
Xiphinema	2.2	3.5	31.9	25.3	4.9	7.8	3.6	6.8	6.4	0.003 **
Predators										
Ironus	0	0	0.9	2.2	1.2	2.0	51.3	55.0	5.1	0.09 **
Mylonchulus	8.8	21.6	58.4	50.1	4.7	6.9	14.8	19.3	4.3	0.01 *
Paractinolaimus	18.6	42.0	6.8	7.3	1.1	1.7	4.2	10.4	0.7	0.54
Tobrilus	0.6	1.4	0.5	1.3	8.0	8.0	1.4	2.3	4.4	0.1*
Omnivores										
Crassolabium	3.7	4.2	18.1	25.3	58.8	45.7	7.9	17.1	5.0	0.009**
Eudorylaimus	182	154	111	105	180	115	65	119	1.2	0.32
Mesodorylaimus	202	211	276	155	7.2	8.8	91	51	4.8	0.01 *
Oriverutus	155	137	234	214	31.8	30.8	31.1	76	3.3	0.04 *
Prodorylaimium	100	107	11.6	28.5	0	0	2.6	6.4	4.5	0.14*
Sectonema	9.3	2.7	25.7	30.7	21.4	13.0	2.8	6.9	2.3	0.10

3.2. Nematode community composition

The community composition of nematodes differed between primary forest and intensive agriculture (F[4,18]=4.24, p≤ 001) with communities of 2 forests and slash and burn grouping in between. (Fig. 2)

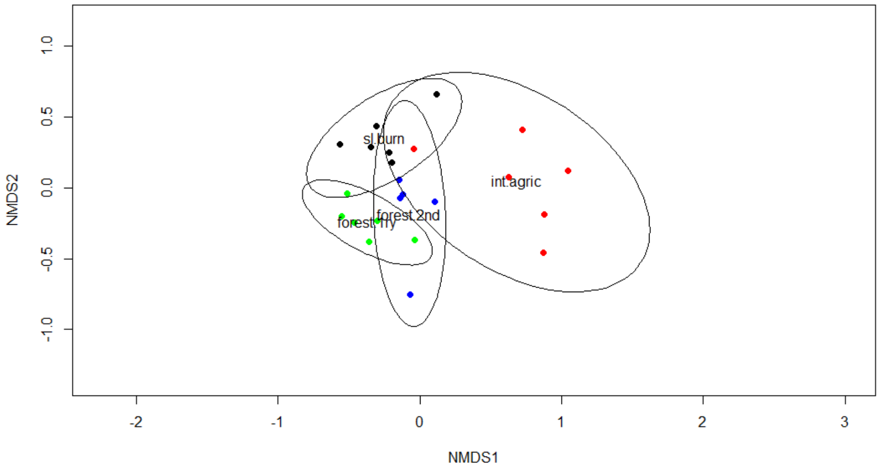

Figure 2. NMDS plot of nematode communites from mineral soils. LUI had a strong impact on the composition of nematode communities. (95% conf. interval) on Bray-Curtis distances of species data F[4,18]=4.24, p≤ 001)

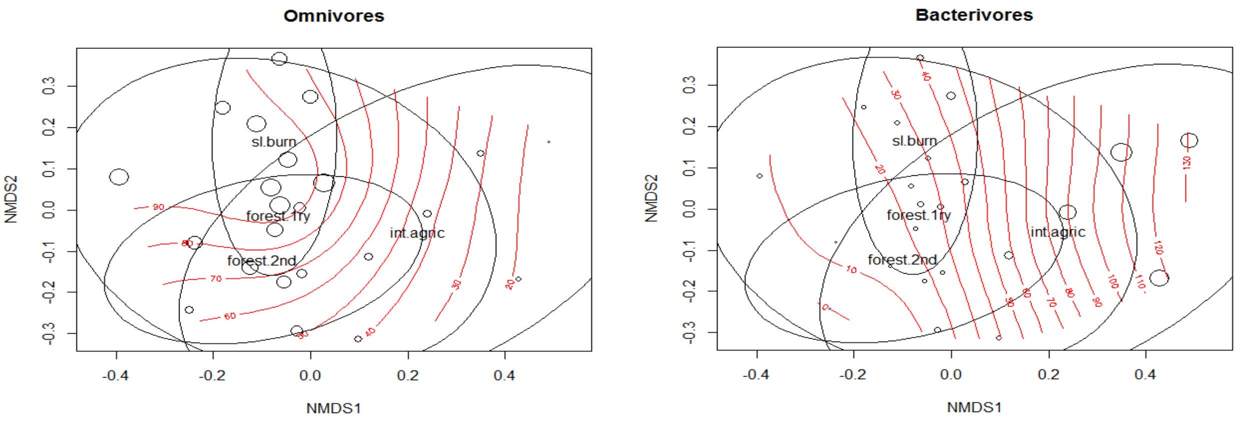

Figure 3. Omnivores dominated in primary forests, and bacterivores in intensively managed agricultural land in Cao Bang

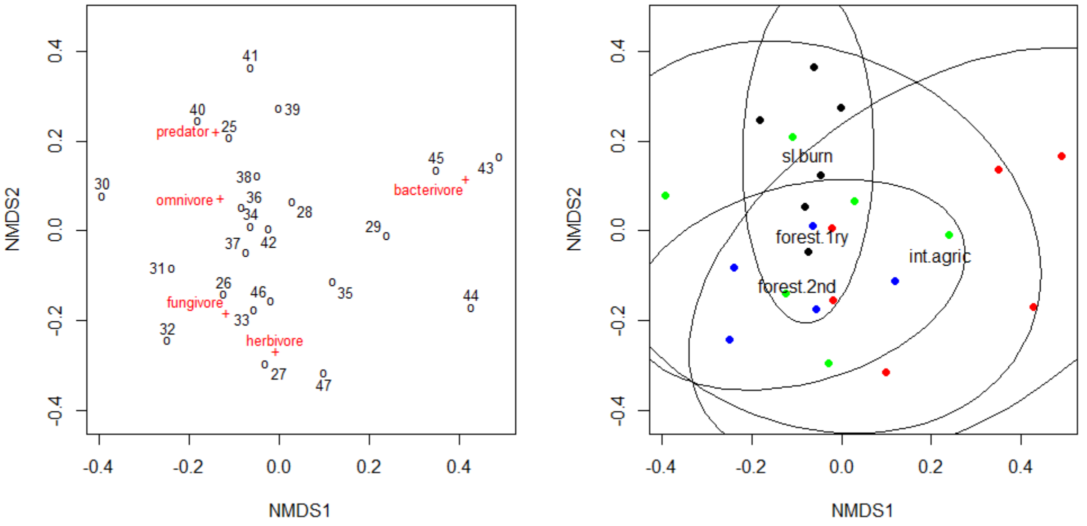

Figure 4. Omnivores dominated in primary forests, and bacterivores in intensively managed agricultural land in Cao Bang

3.3. Changes in soil nematodes community indices

Comparing values of nematode diversity indices among the four different habitat types, significant differences were found in genus richness (S), Shannon and J index, and Eveness. Genus richness was significantly (P< 0.05) higher in the intensive agriculture, followed by slash and burn, secondary forest and it was lowest in primary forest. The Shannon diverisity and the Eveness index showed a similar trend where diversity was highest in intensive agriculture followed by slash and burn. In primary forest, the Shannon and Eveness index was lowest (Table 3)

Table 2: Nematode fauna diversity indices in diferent land use intensity. Values are mean with ± S.E (n=6). F and P values of the effect of LUI are presented. (Significant of effect: P< 0.5: ‘ ’; P < 0.1, ‘*’; p< 0.05 ‘**’; p< 0.001 ‘***’).

Indices	Primary forest (n=6)	Secondary forest (n=6)	Slash and burn (n=6)	Intensive Agriculture (n=6)	F	P
Total abundance	1160±426 a	1221±675 a	648±257 a	1078±335 a	1.98	0.15
J.index	0.7±0 b	0.8±0 ab	0.8±0 ab	0.8±0 a	3.377	04*
Shannon.index	2.0±0.2 a	2.3±0.2 ab	2.4±0.2 bc	2.6±0.2 c	10.85	0001 ***
Species richness	15.7±2.9 c	19.3±2.1 bc	21.3±4.8 ab	26.3±2.9 a	10.92	0001 ***

Maturity Index of the nematode communities in Cao Bang exactly reflected a strong gradient of increasing disturbance of sites with increasing land use intensity. The maturity index (MI) was significantly ($P<0.05$) different among land use types. The highest MI was recorded in the forests (primary and secondary) (4.0) while the lowest was in intensive agriculture (2.9). MI was higher in the forests compared to slash and burn. The maturity index MI25 (omit the cp 1 species) was also similar with MI, higer in natural forest when compared to other land use types. Plan parasitic index was also variable among the land use type, being higest in the primary forest and lowest in the intensive agriculture. Plant Parasitic Index reflected a shift towards root- endo parasitic nematode taxa with increasing agricultural land use (Fig. 5, Table 3).

The Channel Index was also variable among the land uses, the highest value was in slash and burn (85) and it was lowest in intensively cultivated land (48). Channel Index showed a strong shift towards the bacteria-based decomposer channel in agricultural sites compared to a more stable energy transfer through the 'slow' fungal decomposition channel in forest habitats (Fig. 5)

Table 3. Nematode fauna ecological indices in diferent land use intensity. Values are mean with ± S.E (n=6). F and P values of the effect of LUI are presented. (Significant of effect: $P< 0.5$: ‘ ’; $P < 0.1$, ‘*’; $p< 0.05$ ‘**’; $p< 0.001$ ‘***’).

Indices	Primary forest (n=6)	Secondary forest (n=6)	Slash and burn (n=6)	Intensive Agriculture (n=6)	F	P
MI	4.0±0.1	4.0±0.1	3.8±0.2	2.9±0.6	17.89	<001 ***
MI25	4.0±0.1	4.0±0.1	3.8±0.2	3.1±0.5	15.61	<001***
Plant Parasitic Index	4.0±1.7 a	4.1±0.7 a	3.6±1.0 a	3.3±0.8 a	0.695 a	0.5
Chanel Index	60±56.6 a	82.2±39.8 a	85.4±32.5 a	48.2±40.8 a	1.0	0.4
Enrichment Index	15.3±22.6	45.9±26.3	25.0±17.6	61.0±21.1	4.87	0.1 *
Structure Index	99.4±0.5	99.2±0.5	97.0±3.6	79.0±12.6	13.52	<001***

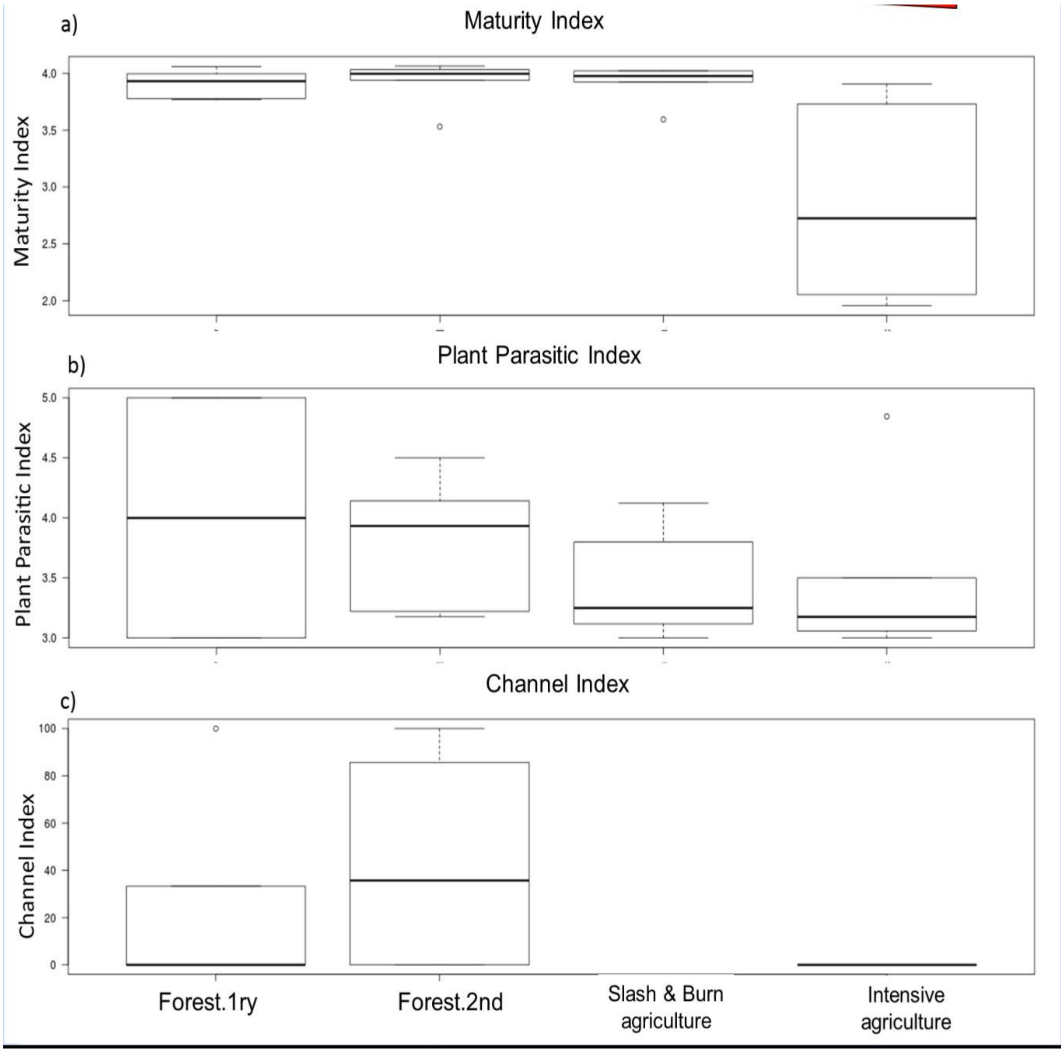

Figure 5. Indicator analyses of nematode communities in mineral soils along a land-use gradient in Cao Bang. a) Maturity Index clearly indicates the high disturbance of sites with intensive agriculture; b) Plant Parasitic Index reflects a shift towards root-endo parasitic nematode taxa with increasing LUI; c) High Channel Index in forest habitats indicates a higher proportion of energy transformed through the 'slow' fungal decomposition channel compared to agricultural sites.

3.4. Soil C/N ratio.

The soil C/N ratio did not change significantly with increasing land-use (Fig. 6)

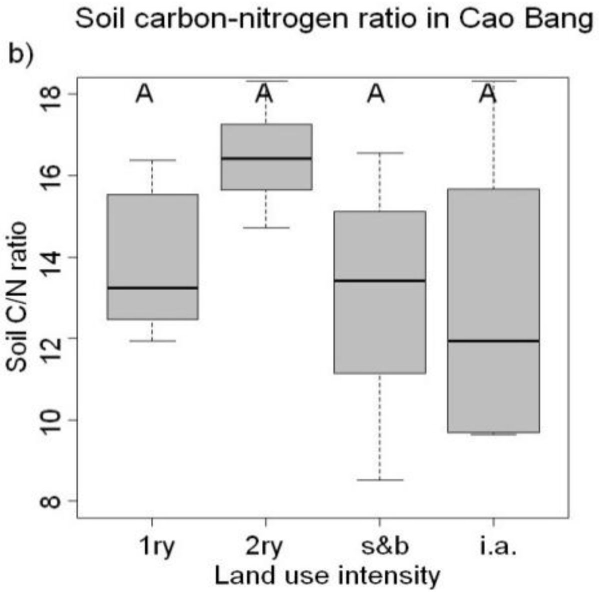

Figure 6. The soil C/N ratio did not change significantly with increasing land-use

4. Discussion

Global nematode diversity patterns are equivocal and certainly much higher than currently proposed (Yeates et al., 2009). The number of known species is consisting a proportion of the true diversities as many new forms are described every year. Several studies show that tropical ecosystems have a lower nematode diversity than temperate region (Procter, 1984; Groombridge, 1992; Giller, 1996; Maraun et al., 2007), while other studies have reported in tropical rain forest in India and Cameroon with relatively higher nematode diversity (Pradhan & Dash, 1987; Bloemers et al., 1997). They recorded 204 morphospecies from 1009 individuals, and estimated and average samples contain 72 species. Based on morphological taxonomic identification revealed so far, our study showed a relatively high diversity of free-living terrestrial nematodes from Vietnam on genus level. In total, more than 4.800 specimens were identified belonging to 77 genera. Our results had twofold higher than study of Zhang et al, 2012 and equal with study of Price and Siddiqi (1994).

References

Achard F., 2002. Determination of deforestation rates of the world's humid tropical forests. Science 297, 999–1002.

Andrássy, I. (2009). *Free-living nematodes of Hungary. III.* Pedozoologica Hungarica nº 5. Hungarian Natural History Museum. Budapest, Hungary. 608 pp.

Ahmad, W. & Jairajpuri, M.S., 2010. Monochida. The predaceous nematodes*. Nematology monographs and perspectives, vol.* 7. E. J. Brill, Leiden – Boston, The Netherlands, 298 pp.

Averyanov, L.V, Phan, L.K., Nguyen H.T., Harder, D.K., 2003. Phytogeographic review of Vietnam and adjacent areas of Eastern Indochina. Komarovia 3, 1–83.

Baermann G., 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneesk. Tijdschr. Ned-Indië, 57, 131–137.

Bloemers, G.F., Hodda, M., Lambshead, P.J.D., Lawton, J.H., Wanless, F.R., 1997. The effects of forest disturbance on diversity of tropical soil nematodes. Oecologia 111, 575–582.

Boag, B., Yeates G.W., 1998. Soil nematode biodiversity in terrestrial ecosystems. Biodiversity and Conservation 7, 617–630.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. TREE 14, 224–228.

Corlett, R.T., Primack, R.B., 2006. Tropical rainforests and the need for cross-continental comparisons. Trends in Ecology & Evolution 21, 104–110.

De Grisse, A., 1969. Redescription ou modifications de quelques techniques utililisées dans l'etude des nematodes phytoparasitaires. Mededelingen van de Rijksfaculteit Landbouwwetenschappen Gent 34, 351–369.

de Mesel, I., Derycke, S., Moens, T., van der Gucht, K., Vinx, M., Swings, J., 2004. Top down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environmental Microbiology 6, 733–744.

de Ruiter, P.C., Neutel, A.M., Moore, J.C., 1998. Biodiversity in soil ecosystems: the role of energy flow and community stability. Applied Soil Ecology 10, 217–228.

Dirzo, R., Raven P.H., 2003. Global state of biodiversity and loss. Annual Review of Environment and Resources 28, 137–167.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics:extension of the nematode faunal analysis concept. Applied Soil Ecology 18, 13–29.

Flegg, J.J.M., 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. Annals of Applied Biology 60, 429–437.

Giller, P.S., 1996. The diversity of soil communities, the “poor man’s tropical rainforest”. Biodiversity and Conservation 5, 135–168.

Grainger, A., 2010. Uncertainty in the construction of global knowledge of tropical forests. Progress in Physical Geography 34, 811–844.

Groombridge, B., 1992. *Global biodiversity: status of the Earth’s living resources*. London, UK, Chapman & Hall.

Hansen, M.C., DeFries, R.S., 2004. Detecting long-term global forest change using continuous fields of tree-cover maps from 8-km advanced very high resolution radiometer (AVHRR) data for the years 1982–99. Ecosystems 7, 695–716.

Kim, K.C., Byrne, L.B. 2006. Biodiversity loss and the taxonomic bottleneck: emerging biodiversity science. Ecological Research 21, 794–810.

Korthalas, G.W., van de Ende, A., van Megen, H., Lexmond, T.M., Kammenga, J.E., Bongers, T., 1996. Short-term effects of cadmium, copper, nickel, and zinc on soil nematodes from different feeding and life-history strategy groups. Applied Soil Ecology 4, 107–117.

Lawton, J.H., Bignell, D.E., Bloemers, G.F., Eggleton, P., Hodda, M.E., 1996. Carbon flux and diversity of nematodes and termites in Cameroon forest soils. Biodiversity Conservation 5, 261–273

Maraun, M., Schatz, H., Scheu, S., 2007. Awesome or ordinary? Global diversity patterns of oribatid mites. Ecography 30, 209–216.

Moens, T., Dos Santos, G.A.P., Thompson, F., Fonsêca-Genevois, V., de Mesel, I., 2005. Do nematodes mucus secrections affect bacterial growth?. Aquatic Microbial Ecology 40, 77–83.

Porazinska, D.L., Giblin-Davis, R.M., Esquivel, A., Powers, T.O., Sung, W., Thomas, W.K., 2010. Ecometagenetics confirm high tropical rainforest nematode diversity. Molecular Ecology 19, 5521–5530.

Porazinska, D.L., Giblin-Davis, R.M., Powers, T.O., Thomas, W.K., 2012. Nematode spatial and ecological patterns from tropical and temperate rainforests. PloS ONE 7, e44641.

Powers, T.O., Neher, D.A., Mullin, P., Esquivel, A., Giblin-Davis, R.M., Kanzaki, N., Stock, S.P., Mora, M.M., Uribe-Lorio, L., 2009. Tropical nematode diversity: vertical stratification of nematode communities in a Costa Rican humid lowland rainforest. Molecular Ecology 18, 985–996.

Powers, T.O., Neher, D.A., Mullin, P., Esquivel, A., GiblinDavis,R.M.,Kanzaki,N.,Stock,S.P.,Mora,M.M., Uribe-Lorio, L., 2009. Tropical nematode diversity: vertical stratification of nematode communities in a Costa Rican humid lowland rainforest. Molecular Ecology 18, 985–996.

Pradhan, G., Dash, M., 1987. Distribution and population dynamics of soil nematodes in a tropical forest ecosystem from Sambalpur, India. Proceedings of the Indian Academy of Sciences (Animal Sciences) 96, 395–402.

Price, N.S., Siddiqi, M.R., 1994. Rainforest nematodes with particular reference to the Korup National Park, Cameroon. Afro-Asian Journal of Nematology 4, 117–128.

Procter, D.L.C., 1984. Towards a biogeography of free-living soil nematodes. I. Changing species richness, diversity and densities with changing latitude. Journal of Biogeography 11, 103–117.

Rockström, J., Steffen, W., Noone, K., Persson, A., Chapin, F.S., Lambin, E.F., Lenton, T.M., Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A., Hughes, T., van der Leeuw, S., Rodhe, H., Sorlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley J.A., 2009. A safe operating space for humanity. Nature 461, 472–475.

Sala, O., Chapin, F., Armesto, J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L., Jackson, R., Kinzig, A., Leemans, R., Lodge, D., Mooney, H., Oesterheld, M., LeRoy Poff, N., Sykes, M., Walker, B., Walker, M., Wall, D., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.

Shearman, P., Bryan, J., Laurance, W.F., 2012. Are we approaching 'peak timber' in the tropics? Biological Conservation 151, 17–21.

Sodhi, N.S., 2008. Tropical biodiversity loss and people - A brief review. Basic and Applied Ecology 9, 93–99.

Sohlenius, B., 1980. Abundance, biomass, and contribution to energy flow by soil nematodes in terrestrial ecosystems. Oikos 34, 186–194.

Sterling, E.J., Hurley, M.M., Le, M.D., 2006. *Vietnam: A Natural History*. Yale University Press, New Haven, pp. 1–448.

Tetuna, M., Ferris, H., 2004. Relationship between nematode life-history classification and sensitivity to stressor: ionic and osmotic effects of nitrogenous solutions. Journal of Nematology 36, 85–94.

Traunspurger, W., Bergtold, M., Goedkoop, W. 1997. The effect of nematodes on bacterial activity and abundance in a freshwater sediment. Oecologia 112, 118–122.

Yeates, G.W., Bongers, T., 1999. Nematode diversity in agroecosystems. Agriculture, Ecosystems & Environment 74, 113–135.

Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in nematode families and genera: an outline for soil ecologists. Journal of Nematology 25, 315–331.

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Appendix

Appendix 1. Mean relative abundance of nematode genera (%) in soils at different land use intensity.

Nematode genera		C-p[a] Value	P-P[b] Value	Land use type			
				Primary forest	Secondary forest	Slash and burn	Intensive agriculture
Herbivores-ectoparasites							
1	*Amplimerlinius*	0	3	0.0	0.0	0.3	0.3
2	*Axonchium*	0	5	0.0	0.0	0.0	1.1
3	*Belondira*	0	5	0.2	0.3	0.3	1.3
4	*Criconema*	0	3	0.0	0.0	0.0	0.1
5	*Discocriconemella*	0	3	0.0	0.0	0.5	0.1
6	*Longidorus*	0	5	0.0	4.9	0.0	0.0
7	*Trichodorus*	0	4	0.0	0.0	0.1	0.0
8	*Xiphinema*	0	5	0.2	2.2	0.3	0.3
Herbivores-semi endo parasites							
9	*Helicotylenchus*	0	3	0.0	1.9	0.7	2.2
10	*Rotylenchus*	0	3	0.0	0.1	0.0	0.8
11	*Scutellonema*	0	3	0.0	0.0	0.0	0.1
Herbivores-migratory endo parasites							
12	*Hirschmanniella*	0	3	0.0	0.0	0.0	0.9
Herbivores-epidermal/root hair feeder							

13	*Psilenchus*	0	2	0.0	0.0	0.2	1.3
14	*Tylenchus*	0	2	0.2	0.9	0.7	0.9
Fungivores							
15	*Aphelenchoides*	2	0	0.4	1.8	0.7	7.2
16	*Aphelenchus*	2	0	0.0	0.0	0.1	4.4
17	*Dorylaimoides*	4	0	4.3	1.8	0.0	0.2
18	*Filenchus*	2	0	0.0	0.0	0.3	3.8
19	*Funaria*	4	0	0.0	0.0	0.1	0.0
20	*Leptonchus*	4	0	0.0	0.5	0.4	0.3
21	*Proleptonchus*	4	0	0.0	0.3	0.0	0.0
22	*Tylencholaimellus*	4	0	0.0	0.8	0.0	0.1
23	*Tylencholaimus*	4	0	4.6	0.1	0.0	0.0
Bacterivores							
24	*Acrobeles*	2	0	0.0	0.0	0.0	0.1
25	*Acrobeloides*	2	0	0.0	0.0	0.0	1.0
26	*Alaimus*	4	0	15.9	4.8	4.3	3.8
27	*Amphidelus*	4	0	2.2	0.0	1.0	1.9
28	*Anaplectus*	2	0	0.0	0.0	1.3	0.3
29	*Aphanolaimus*	3	0	0.0	0.0	0.1	0.3
30	*Bicirronema*	1	0	0.1	0.3	0.0	0.0
31	*Caenorhabditis*	1	0	0.1	0.1	0.0	1.1
32	*Cephalobus*	2	0	0.0	0.0	0.1	0.0
33	*Chiloplacus*	2	0	0.0	0.0	0.0	0.3
34	*Daptonema*	2	0	1.4	0.5	0.3	0.1
35	*Eucephalobus*	2	0	0.0	0.0	0.1	6.7
36	*Geomonhystera*	2	0	0.0	0.0	0.1	0.1
37	*Mesorhabditis*	1	0	0.0	0.0	0.1	2.7
38	*Microlaimus*	3	0	0.0	0.0	0.0	0.3
39	*Panagrolaimus*	1	0	0.0	0.0	0.0	1.7
40	*Prismatolaimus*	3	0	0.0	0.0	0.1	0.1
41	*Pseudacrobeles*	2	0	0.0	0.0	0.1	1.0
42	*Steinernema*	1	0	0.0	0.0	0.0	0.2
43	*Theristus*	2	0	0.4	0.0	0.4	0.5
44	*Wilsonema*	2	0	0.0	0.0	0.4	0.0
Predators							
45	*Clarkus*	4	0	1.8	0.1	0.2	0.0
46	*Cobbonchus*	4	0	0.0	0.0	0.3	0.1
47	*Iotonchus*	4	0	0.2	1.2	3.6	3.2
48	*Ironus*	4	0	0.0	0.1	0.1	3.6
49	*Laevides*	5	0	0.0	0.0	0.0	0.1
50	*Mononchus*	4	0	0.0	0.6	0.2	0.4
51	*Mylonchulus*	4	0	0.5	4.1	0.3	1.0
52	*Oxydirus*	5	0	0.0	0.0	0.0	0.2
53	*Paractinolaimus*	5	0	1.3	0.5	0.1	0.3
54	*Prionchulus*	4	0	0.7	0.3	0.7	0.0
55	*Tobrilus*	3	0	0.1	0.0	0.6	0.1
56	*Trypila*	3	0	0.0	0.3	0.4	0.2
Omnivores							
57	*Actinolaimus*	4	0	0.0	0.0	0.1	0.0

58	*Aporcelaimellus*	5	0	0.0	0.1	0.2	0.4
59	*Aporcelaimus*	5	0	0.0	0.2	0.0	0.5
60	*Aporcelinus*	5	0	0.0	0.0	0.0	0.6
61	*Coomansus*	4	0	4.0	0.0	0.0	0.1
62	*Crassolabium*	4	0	0.4	1.3	4.1	0.6
63	*Dorylaimus*	4	0	0.3	0.0	2.7	2.7
64	*Enchodorus*	4	0	3.9	2.0	0.4	0.0
65	*Epidorylaimus*	4	0	1.1	0.1	0.7	0.0
66	*Eudorylaimus*	4	0	15.2	7.8	12.6	4.6
67	*Labronema*	4	0	0.0	5.0	0.6	0.2
68	*Labronemella*	4	0	0.0	0.2	0.1	0.0
69	*Mesodorylaimus*	4	0	17.6	19.4	0.5	6.4
70	*Mylodiscus*	4	0	0.0	0.0	0.0	0.1
71	*Opisthodorylaimus*	5	0	0.3	2.3	0.0	0.5
72	*Oriverutus*	4	0	14.1	16.5	2.2	2.2
73	*Prodorylaimium*	4	0	7.6	0.8	0.0	0.2
74	*Prodorylaimus*	4	0	0.2	0.0	0.0	0.0
75	*Rhyssocolpus*	4	0	0.0	0.0	0.0	0.1
76	*Sectonema*	5	0	1.0	1.8	1.5	0.2
77	*Tyleptus*	4	0	0.2	0.1	0.1	0.0

[a]Colonizer-persister scale 1-5 where cp 1 are colonizers characterized by short generation time and cp 5 are persisters characterized by long generation time (Bonger, 1990).

4. Discussion

4.1 Species richness of free-living nematodes in tropical forest

Global nematode diversity patterns are ambigous and the species richness is certainly much higher than currently proposed (Yeates *et al*. 2009). The number of known species accounts for only a small proportion of existing taxa as many new forms are described every year.

Several light microscopy studies indicated that tropical ecosystems have a lower nematode diversity than temperate regions in Europe (Procter 1984; Groombridge 1992; Giller 1996; Maraun *et al*. 2007), while other studies have reported relatively higher nematode diversity in tropical rain forest in India and Cameroon (Pradhan & Dash 1987; Bloemers *et al*. 1997). Price & Siddiqi (1994) found 119 genera of nematodes in 23 samples taken from the rooting zones of trees in the Koroup National Park, Cameroon while an investigation of a temperate forest in China by Zhang *et al.* 2012 identified only 62 genera. However comparisons of tropical and temperate studies must keep in mind the high variation in results from tropical systems. Based on morphological taxonomic identifications revealed so far, our study showed a relatively high diversity of free-living terrestrial nematodes from Vietnam on the genus level. In total, more than 14.000 specimens were identified (see. Appendix 1). These nematodes belonged to 105 genera, 42 families and 9 orders. Which studies are giving a more accurate account? First it is important to examine the differences caused by methodological artefacts. The number of studies from the tropics is far lower, so it is difficult to establish a meaningful consensus. Some studies use Bearmann funnel techniques (Baermann 1917) (base on avtive migration of nematodes), others use sugar centrifugation (Freckeman & Virginia 1993) (based on passive separation due to density differences of nematode and soil particles) or the Oostenbrink elutriator (Oosternbrink 1960) techniques. As centrifugation includes dead nematodes it will likely yield higher numbers than Bearmann funnel collections or other methods that rely on active nematodes (McSorley & Parrado 1987; McSorley & Walter 1991; McSorley & Frederick 2004). Most studies from temperate regions utilise the Osternbring technique which is more efficient but also more expensive to establish, while in the tropics, and in our study, modified Bearmann funnel techniques are employed. In Bearmann funnel approaches it is usually only possible to extract nematodes from 10 grams of soil, but modifications of this approach use a larger surface area from which the nematodes can be extracted, increasing the numbers found. Zhang *et al.* 2012 utilised a modified cotton wool approach which is essentially similar to the modified Bearmann funnel approach we used, so the higher yield from tropical soils in our study is in this context robust. Some studies focus only on the litter or mineral layer of the soil, whereas others include both. It has been shown that for nematodes, like other soil organisms, large differences in richness occur between the first 10cm and lower soil layers. In some cases, such as in intensive agriculture, litter layers are not present. It is therefore important that only litter layer and mineral soil richnesses are directly compared. Many studies of both temperate and tropical soils collected a small number of samples, often 24 samples (Bloemer *et al.* 1997), and do not include species accumulation curves to give an indication of how many new species would be found with increased sampling affact. It is not clear to what extent scale and and sampling interrelate, changes in the richess of nematodes could occur at smaller scales, based on habitat heterogeneity. Whether sampling regimes were conducted according to local patterns of species richness is not clear, in some cases distant locations are chosen and in others samples are taken close by one another. Most studies identify the nematodes to genus level (Groombridge 1992; Giller 1996; Neher *et al*. 2005; Zhang *et al.* 2012), in some cases only to family level (Neher *et al*. 1995). Often particular groups, such as the order Dorylamida are left out due to the difficulties of their morphological identification. In our case, we made a closer inspection of the Dorylamida, down to species level, so that we could really gain species richness estimations for the tropics. The incredible new diversity we identified at species level gives some indication of the weakness in comparisons to studies estimating richness at the genera level.

This study is the first contribution to our understanding of free-living terrestrial nematodes diversity in limestone karst tropical forests of Vietnam. The Dorylaimida is probably the most diverse nematode order as they make up nearly 50% of the total nematode genera. Our morphological study recorded **48 genera** and **13 families** of the Order Dorylaimida, more than ten, five and three times the number of genera found by the study from Andrássy (1970), Nguyen (2007) and Nguyen (2011), respectively. The nematode fauna in 12 plots (primary and secondary forests) in Huu Lien Nature Reserve, Lang Son Province in Vietnam was examined. Nematodes in the order Dorylaimida were identified to species level in order to get an estimate on the numbers and diversity of known and undescribed taxa. More than 300 specimens of 30 species, 20 genera and 9 families have been examined and characterized. The catalogue of the species shows that its nematode fauna consists of four major components. The dorylaimid fauna of this area consisted of a few (8%) cosmopolitan species (*Aporcelaimellus obtusicaudatus, Tylencholaimus teres* and *Mesodorylaimus clavicaudatus*), some (8%) Paleotropical forms (*Longidorella xenura, Proleptonchus aestivus* and *Tyleptus projectus*), a series (51%) of species which presumably belong to the Oriental realm/region (*Axonchium thoubalicum, Belondira murtazai, Labronema glandosum, L. neopacificum, Oriverutus parvus, Oxybelondira paraperplexa...*), and a good number (33%) of forms belonging to non-described species of the genera *Aporcelinus, Aporcelaimoides, Oriverutus* and *Sectonema*. It is especially remarkable that more than one-third of the dorylaimids species examined certainly represent unknown taxa, a proof of the much understudied nematode diversity in the poorly explored natural areas of Southeast Asia.

The study of Vietnamese dorylaims allows not only their characterization and identification but, very often, to obtain new relevant data on the taxonomy including discovery of new taxa. The most remarkable cases refer to the genera *Aporcelaimoides* Heyns*, Aporcelinus* Andrássy*, Sectonema* Thorne*.*

The genus *Aporcelaimoides* is an interesting aporcelaimoid taxon, which was erected by Heyns (1965) to accommodate two new species namely *A. probulbum* (type species) and *A. californicum.* Thirty year later, Siddiqi (1995) regarded it as a junior synonym of *Sectonema* and in 2009, Andrássy also followed Siddiqi's opinion. Therefore, the identity of *Aporcelaimoides* has been a matter of some controversy. Very recently, Peña-Santiago & Álvarez-Ortega (2014) redescribed *S. ventrale* the type species of *Sectonema*, and concluded that "the protrusible structure of *Sectonema*, as observed in its type species, is not a typical mural tooth as seen in nygolaims, but a reduced odontostyle with its base occupying most (if not whole) the stomatal lumen". It means that mural odontostyle of *Aporcelaimoides* significantly differs from the reduced odontostyle of *Sectonema*. Unfortunately, there is no molecular information available to confirm the morphological data. With three new species from Vietnam: *Aporcelaimoides brevistylum*, *A. minor*, *A. silvaticum* and three species transferred from *Sectonema*, **the genus *Aporcelaimoides* was restored as valid genus** making a total of 8 valid species in the genus *Aporcelaimoides* (See pulication 2).

Thorne (1930) classified *Sectonema* new genus under the family Dorylaimidae. Later (1935), the same author created the subfamily Nygolaiminae under Dorylaimidae to accommodate the genera *Nygolaimus* and *Sectonema.* Heyns (1965) proposed the new family

Aporcelaimidae to accommodate the genus *Sectonema* and revised a genus *Sectonema*, with ten valid species. Fifty years after, the classification of *Sectonema* under Aporcelaimidae has not been modified. Nevertheless, new species were subsequently described or transferred to *Sectonema* by Altherr (1965), Jairajpuri & Baqri (1966); Popovici (1978); and Siddiqi (1984, 1995), making the total current number of valid species to 24. More recently, Peña-Santiago & Álvarez-Ortega (2014) re-described *S. ventrale*, the type species of the genus, providing new relevant information about the nature of the protruding stomatal structure, rather than a reduced axial odontostyle than a mural tooth. We recoreded five new species of the genus *Sectonema*: *S. birrucephalum, S. buccociliatum, S. caobangense, S. ciliatum*, *S. tropicum* and *S. Vietnamense* and provided the molecular phylogeny tree for *S. caobangense, S. ciliatum*, *S. tropicum* (see publications 3, 4 and 7)

Andrássy (2009a) proposed the new genus *Aporcelinus* to accommodate two new species, *A. altitudinalis* and *A. mediterraneus*, as well seven previously known than were transferred from *Allodorylaimus*. *Aporcelinus* was originally classified under Aporcelaimidae, Aporcelaimellinae, compared to *Aporcelaimellus, Makatinus* and *Allodorylaimus* in Qudsianematidae, Qudsianematinae. It was separated from *Aporcelaimellus* and *Makatinus* by its thinner cuticle at tail (*vs.* distinctly thickened), absence (*vs.* presence) of cervical lacunae, presence (*vs.* absence) of a lobe at pharyngo-intestinal junction, presence (*vs.* absence) of hiatus, and conical (*vs.* short and rounded to conical) tail with pointed (*vs.* rounded tip if the tail is conical); and from *Allodorylaimus* in its three-layered (*vs* two-layered) cuticle, guiding ring plicate (*vs* typical dorylaimoid) and tail not ventrad curved (*vs.* distinctly curved ventrad). More recently, Vinciguerra *et al.* (2014) raised some doubt about the classification of *Aporcelinus* under Aporcelaimidae due to its features also shared with members of Qudsianematidae. We recored three new species of the genus *Aporcelinus*: *A. falcicaudatus, A. paramamillatus, A. paraseychellensis,* making the total number of *Aporcelinus* species to 24 valid species (see publications 6, 8).

The discussion of the taxonomy/systematics of these three genera is an intricate matter and particularly challenging because it is highly diverse and species show only little differences in morphological characters.

Furthermore, we also recorded new data of three rare belondirid species from Vietnam with the first record and description of the male of *Oxybelondira paraperplexa* Ahmad & Jairapuri (see Publication 1). Based on the new evidence of the SEM pictures (Fig. 3 in Publication 5), we re-described of *Cephalobus topali* Andrássy and transfered to *Acrobeloides* (Cobb).

In additionally, comparative genetic surveys of nematode richness in tropical and temperate regions have only just started. Databases of sequences of nematodes from tropical regions are still extremely poor. Comparisons therefore are very likely to find little overlap, as only a small number of species from each location are detected. A recent study by Porazinska *et al*. (2012) found almost no nematode species shared between a temperate and tropical forest, in the United States of America and Costa Rica, though in light of how little species are known from either location this not surprising. In our study, we sequenced 67 dorylamid individuals, however of these 67 different sequences only 5 could be matched with those from the Genebank database at the 95% level (Fig. 1, Publiaction 9), which is only proxy for even genus level differences. Porazinska *et al*. (2012) did however also find a threefold higher species richness in the tropical samples than in the temperate. Comparisons between different tropical habitats are for same reasons also problematic. Porazinska *et al*. (2012) found 214 observed species whereas another study conducted in Costa Rica by Powers *et al*. (2009) recored 167 observed species. Both studies found that in a tropical rainforest of Costa Rica, 66% and 80% respectively, of the overall nematode genetic diversity existed in the litter and understory habitats (based on MOTU and OCTU, respectively. We found that mineral soil habitats can also harbor a high species richness and more studies are required to assert whether soil or litter in the tropics contain a higher number of species.

In conclusion, the diversity of soil nematodes in Vietnam was extremely underestimated and hypothesis 1 was confirmed.

4.2 Impact of land use intensity on nematode communities

Nematode community composition

A Non-Metric Multidimensional Scaling analysis indicated that nematode community composition differed between regions and different land-use intensity levels. The nematode community composition in Cao Bang, Cat Ba and Lang Son (Fig. 6) differed, suggesting an affect of geographical distribution although these localitions have similar limestone habitats. We expected that the nematodes from Cat Ba Island would differ from the others because the Island is isolated. However, our statistical analysis distinguishes the nematode community in Cao Bang from the other two locations. The forest in Cao Bang is more pristine, with less historical anthropogenic disturbance, and the average elevation of the areas sampled there is higher than in the other locations. Even within the subtropical forest habitats in these three locations, we see such large differences in the nematode community. The geographical barrier of the sea is of course impermanent as the sea level fluctuates, but it is interesting to see that these historical effects have perhaps less impact in our case.

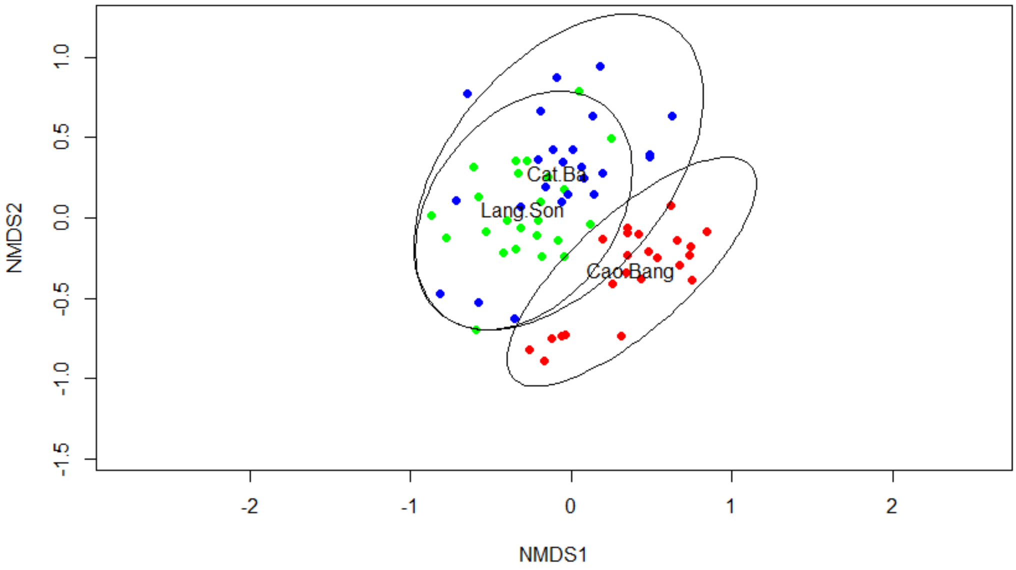

FIGURE 6. NMDS plot of nematode communities at genus level in the upper layer of mineral soils show clear differences in community composition between three regions: Cao Bang and Cat Ba and Lang Son (ellipses show 95% conf. interval)

Our study clearly indicated that the species richness and the divesity in Cao Bang in the primary forest were higher than in the secondary forest and in the intensive agricultures. That could be due to several factors: 1) the soil in the primary forest was not disturbed. This stable evironment provides a suitable habitat for some specialist K-strategy nematode genera. 2) The higher diversity of vegetation in the forest may be linked to the soil nematode abundance and diversity. 3) Agricultural intensification is usually associated with increased disturbance of the soil through tillage, indiscriminate use of mineral fertilizers and pesticides, manipulation of organic residues and planting, this lead to decrease in the diveristy and species (Yeates *et al*. 1999).

Land-use intensity (LUI) also had distinct impacts on the nematode community (Fig. 2 in Publication 9). With increasing LUI, dominance patterns in nematode communities changed and we observed colonization of new taxa especially the fungivores and the plant parasitic nematodes in intensive agriculture land. Our data clearly indicated the significant different of trophic group (bacterivores, fungivores, omnivore and predator) within the increasing of land use intensity and among the regions.

My study suggested several possible causes that drive soil nematode community structure with the increasing the land use intensity. First, the high number of predator-omnivores component in ther primary forest was one of the important channels of the soil nematode food web (Sanchez-Moreno *et al*. 2011) and the interactions caused by this nematode trophic or functional group was one of the major driving in the below-ground ecosystem. This can strongly affect soil nematode communities (Cesarz *et al*. 2013). Albers *et al*. (2006) suggested that the shift of nematode community from the forest to the intensive agriculture. Further, with different LUI, vegetation properties change in forests and in intensive agricultures, in turn, also influences nematode communities.

Ecological indices

We tested whether different nematode ecological indices could be applied to our tropical soils. There is a lack of comparable studies investigating ecological indices in pristine habitats, and so we were unsure which would likely prove robust. Many of these tests were designed for temperate communities which usually contain a higher proportion of bacteriovores or fungivores than we expected to see in a tropical soil. The MI was developed by Bonger (1990) for temperate systems and assigns values based on an r-k spectrum, where higher trophic groups get higher scores. Studies in temperate regions yielded a MI of 2.01 – 2.13 for conifer forests (de Goede & Dekker 1993), 2.40-2.43 for grasslands (Yeates *et al.* 1997) and 2.01 – 2.13 for intensive agriculture (McSorley & Frederick 1996). In our case, the much higher numbers of omnivores and predators increased the MI dramatically to 4 or 4.11 in subtropical forests or 2.9 in intensive agriculture. The fact that we identify more predators and omnivores is also strongly influenced by our inclusion of the Dorylamida, and contains a high proportion of omnivores and predators. In our case the MI may still function for finding evidence of disturbance in communities, but direct comparisons with temperate systems with entirely differently structured communities may not be possible.

Plant parasitic index was also variable among the land use type, being higest in the primary forest and lowest in the intensive agriculture. In particular Plant Parasitic Index reflected a shift towards root- endo parasitic nematode taxa with increasing agricultural land use.
The Channel Index showed a strong shift towards the bacteria-based decomposer channel in agricultural sites compared to a more stable energy transfer through the 'slow' fungal decomposition channel in forest habitats.

The changes in nematode fauna structure were visualized and indicated the shifts of nematode community from the forest to intensive agriculture (from the stable environment to stress habitats) by the cp- triangules. The result of the shifts in nematode community was indicated that the nematode community in intensive agriculture was expressed by a shift to the upper left, stress to the lower left and the stable environmental to the right. Analysis of data clearly indicated the interpretation of patterns in the nematode community due to the changes in land-used intensity. In comparision with the data from temperate region (de Gooede *et al.* 1993), we can see clearly the different between two communities.

Total abundance nematodes

In our study, the total abundance of nematodes per 100gr of fresh soil showed no significant differences between the land use intensities and the three forests. Though no statistical tests could confirm a difference in the treatments in terms of abundance, the highest recorded abundance was in the primary forest with an average of 1537ind./100gr. These values are quite similar to the 1290ind./100g reported in the mineral soil of a pine forest in temperate region in the Netherlands by de Goede & Dekker (1993). If the abundances of nematodes per gram soil are similar across continents, it emphasizes the higher diversity of nematodes per gram soil in the tropics.

Trophic groups

We found that very high proportion of omnivores and predators were the dominant feeding type (46-74.1%) in primary forest of three regions. Comparing with other groups, Dorylaimida is one of the most diverse order in terms of numbers of genera as well as abundance. Due to the high degree of environment stability of the forests in those regions, which have been undisturbed by human activities. Comparing with other continental, Sohlenius & Wasilewska (1984) also reported that populations of dorylaimids are sensitive to disturbance. Therefore they can be used as indicators of environmental disturbances. One possibility is that they are sensitive to changes in the abundance or community structure of their food resources. Dorylaimids and mononchids may also be more directly sensitive than other nematode groups to disturbance-induced changes and to the physio-chemical conditions of the soil environment. It is also indicated that the resolution of the omnivores and predators nematodes group may appropriate in defining undisturbed soil ecosystem status. Base on the natural morphology of the group and their functional assemblage, so that they can reveal a response to specific pattern of the environmental. As typical K-strategists, omnivores and predators, unlike bacterivores, display more or less similar preferences of habitats. They often adapt with the response slower than other trophic groups therefore they can be especially sensitive to disturbances in soil ecosystems (Bongers & Bongers 1998; Ferris *et al*. 2001). In our study, the most dominant genera of omnivores were recorded in all sites: *Eudorylaimus, Mesodorylaimus, Aporcelaimellus* and *Oriverutus*. *Eudorylaimus* is an omnivore that can exploit a variety of food sources (Yeates *et al*. 1993). Our study strongly compliments with others studies from Florida and Europe (Mcsorley 2012). Ecological studies that included numerical data on these genera were reviewed to determine key aspects of their ecology and behaviour. These three genera: *Eudorylaimus, Mesodorylaimus, Aporcelaimellus* were also the dominant omnivores in many parts of Europe and Florida (USA), and often occurred together. Multiple species within the same genera may be present in one sample and we call this co-occurence species. Although the omnivores were present in high number of all habitats however the greatest numbers were reached in primary forests. We also recorded the dominant predator genera of mononchids group: *Clarkus, Iotonchus, Mylonchulus, Paractinolaimus,* and *Prionchulus.*

The bacterivores were highest 14% in intensive agriculture while the slash and burn contained only 5%. In our study, the most dominant genera of bacterivores were recorded in all habitats: *Alaimus, Amphidielus, Mesorhabditis, Eucephalobus, Panagrolaimus, Theristus,* and *Daptonema.* Ferris *et al*. (1997) provided evidence for different contributions of bacterivorous nematode species to N-mineralization. Different responses of constituent species in the nematode community may indicate their unique and thus critical participation in nutrient and energy flow on a temporary scale.

The fungivores were highest 15% in intensive agriculture while the slash and burn contained only 3%. The pattern formed by fungivorous nematodes was shaped by *Aphelenchus*. Again, higher abundance of *Aphelenchus* overshadowed the response trend formed by

Aphelenchoides and other fungal-feeding nematodes. Lack of data on precise feeding preferences of these nematode species and their contributions to nutrient cycling limits our understanding of the roles of these fungivores. Based on findings of Ferris *et al*. (1997), however, we speculate that fungivores, like bacterivores, differ in their ability to mineralize nitrogen, thus information on the individual genera or even species seems more appropriate.

The herbivores (plant parasitic nematodes) were highest 24% in intensive agriculture while in the primary forest contained only 11%. In particular the plant parasitic nematodes reflected a shift towards root- endo parasitic nematode taxa with increasing agricultural land use**.** We recorded some of the herbivores-ectoparasites genera: *Discocriconemella, Hemicriconemoides, Paralongidorus, Trichodorus* and *Tylenchorhynchus,* some of the herbivores-semi endo parasites: *Helicotylenchus, Hoplolaimus, Rotylenchus, Scutellonema* and herbivores-migratory endo parasites: *Hirschmanniella, Pratylenchus* which only appeared in secondary forest, slash and burn and intensive agrilucture land. In primary forest, only the herbivores-ectoparasites nematodes were recorded: *Axonchium, Belondira, Criconema, Longidorella, Longidorus, Metaxonchium, Oxybelondira,* and *Xiphinema.*

In conclusion, the land use intensity had a strong impact on the nematode communities, the hypothesis 2 was confirmed.

4.3 The relationships of nematode community composition and soil C/N ratio

The C/N ratio analysis showed that soil organic carbon and total nitrogen were relatively not important in influencing soil nematode communities among the soil's environmental factors. However, the proportion of variance explained by soil properties indicated that soil nematode communities are only partially controlled by other environmental factors. The soil C/N ratio did not change significantly with increasing land-use. Therefore, changes in nematode communities along gradients of within increasing land use intensity could not be simply explained by abiotic soil conditions, the hypothesis 3 was not verified.

5. References

Abolafia, J. & Peña-Santiago, R. (2005) Nematodes of the order Rhabditida from Andalucía Oriental: *Pseudacrobeles elongatus* (de Man, 1880) comb. n. *Nematology,* 7, 917–926.

Achard F. (2002) Determination of deforestation rates of the world's humid tropical forests. *Science*, 297, 999–1002.

Ahmad, W. & Sturhan, D. (2000) Description of five new species of Dorylaimida (Nematoda). *International Journal of Nematology,* 10, 55–66.

Albers, D., Schaefer, M. & Scheu, S. (2006) Incorporation of plant carbon into the soil animal food web of an arable system. *Ecology*, 87(1), 235–245.

Altherr, E. (1965) La faune des sables sumergés des rives du Rhin près de Krefeld. Nématodes. *Gewässer und Abwässer, Düsseldorf* , 1965(39/40), 80–101.

Álvarez-Ortega, S., Subbotin, S.A. & Peña-Santiago, R. (2013a) Morphological and molecular characterization of Californian species of the genus *Aporcelaimellus* Heyns, 1965 (Dorylaimida: Aporcelaimidae). *Nematology*, 15, 431–439.

Andrássy, I. (1955) Az erdei talajban élő fonálfégek (Nematoda) mennyiségi és produkciósbiológiai vizgálata. *Kandidátusi értekezés*, Budapest, 260 pp.

Andrássy, I. (1970) Freilebende Nematoden aus Vietnam. *Opuscula Zoologica Budapestinensis*, 10, 5–31.

Andrássy, I. (1976) *Evolution as a basis for the systematization of nematodes*. London, Pitman Publishing. 288 pp.

Andrássy, I. (1988) The superfamily Dorylaimoidea (Nematoda) – a review. The family Dorylaimidae. *Opuscula Zoologica Budapestinensis,* 23, 3–63.

Andrássy, I. (1992) A short census of free-living nematodes. *Fundamental and applied Nematology*, 15, 187–188.

Andrássy, I. (2005) *Free-living nematodes of Hungary. I. Pedozoologica Hungarica nº 5*. Hungarian Natural History Museum. Budapest, Hungary. 518 pp.

Andrássy, I. (2007) Contribution to the genus *Opisthodorylaimus* Ahmad & Jairajpuri, 1982 (Nematoda: Dorylaimida), with description of two new species. *Opuscula Zoologica Budapestinensis,* 36, 3–17.

Andrássy, I. (2009) *Free-living nematodes of Hungary. III. Pedozoologica Hungarica nº 5*. Hungarian Natural History Museum. Budapest, Hungary. 608 pp.

Andrássy, I. (2009a) *Aporcelinus*, a new genus of aporcelaimoid nematodes (Dorylaimida), and its species. *International Journal of Nematology*, 19, 121–136.

Averyanov, L.V, Phan, L.K., Nguyen H.T. & Harder, D.K. (2003) Phytogeographic review of Vietnam and adjacent areas of Eastern Indochina. *Komarovia*, 3, 1–83.

Baermann G. (1917) Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneesk. Tijdschr. *Ned-Indië*, 57, 131–137.

Beare, M.H., Reddy, M.V., Tian, G. & Srivastava, S.C. (1997) Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of decomposer biota. *Applied Soil Ecology*, 6, 87–108.

Bloemers, G.F., Hodda, M., Lambshead, P.J.D., Lawton, J.H. & Wanless, F.R. (1997) The effects of forest disturbance on diversity of tropical soil nematodes. *Oecologia*, 111, 575–582.

Boag, B., & Yeates, G.W. (1998) Soil nematode biodiversity in terrestrial ecosystems. *Biodiversity and Conservation*, 7, 617–630.

Bongers, T. & Bongers, M. (1998) Functional diversity of nematodes. *Applied Soil Ecology*, 10, 239–251.

Bongers, T. & Ferris, H. (1999) Nematode community structure as a bioindicator in environmental monitoring. *Tree,* 14, 224–228.

Bongers, T. (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, 83, 14-19.

Bongers, T. (1999) The Maturity Index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. *Plant and Soil*, 212, 13-22.

Bongers, T., Alkemade, R. & Yeates, G.W. (1991) Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. *Marine Ecology Progress Series,* 76, 135–142.

Bongers, T., Goede, R.G.M. de, Kappers, F.I. & Manger, R. (1989) Ecologische typologie van de Nederlandse bodem op basis van de vrijlevende nematodenfauna. *RIVM-rapport* 718602002

Bongers, T., van der Meulen, H. & Korthals, G. (1997) Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions. *Applied Soil Ecology*, 6, 195–199.

Brook, B.W., Sodhi, N.S. & Bradshaw, C.J.A. (2008) Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, 23, 453–460.

Brusca, R. C & Brusca, G. J. (1990) *Invertebrates*. Sinauer associates, Inc. Publishers. 922pp.

Cesarz, S., Ruess, L., Jacob, M., Jacob, A., Schaefer, M. & Scheu, S. (2013) Tree species diversity versus tree species identity: driving forces in structuring forest food webs as indicated by soil nematodes. *Soil Biology and Biochemistry*, 62, 36–45.

Cock, M.J.W., Biesmeijer, J.C., Cannon, R.J.C., Gerard, P.J., Gillespie, D., Jimenez, J.J., Lavelle P.M. & Raina S.K. (2012) The positive contribution of invertebrates to sustainable agriculture and food security. CAB Reviews: Perspectives in Agriculture, Veterinary Science, *Nutrition and Natural Resources*, 7, 1-27.

Corlett R.T. & Primack R.B. (2006) Tropical rainforests and the need for cross-continental comparisons. *Trends in Ecology & Evolution*, 21, 104–110.

Cox, C.B. (2001) The biogeographic regions reconsidered. *Journal of Biogeography*, 28, 511-523.

D'haeze, D., Deckers, J., Raes, D., Phong, T.A. & Loi, H.V. (2005) Environmental and socio-economic impacts of institutional reforms on the agricultural sector of Vietnam: land suitability assessment for Robusta coffee in the Dak Gan region. *Agriculture, Ecosystems and Environment*, 105, 59–76.

de Goede, R.G.M. & Bongers, T. (1994) Nematode community structure in relation to soil and vegetation characteristics. *Applied Soil Ecology*, 1, 29–44.

de Goede, R.G.M. & Dekker, H.H. (1993) Effects of liming and fertilization on nematode communities in coniferous forest soils. *Pedobiologia*, 37, 193–209.

de Grisse A. (1969) Redescription ou modifications de quelques techniques utililisées dans l'etude des nematodes phytoparasitaires. *Mededelingen van de Rijksfaculteit Landbouwwetenschappen Gent*, 34, 351–369.

de Mesel, I., Derycke, S., Moens, T., van der Gucht, K., Vinx, M. & Swings, J. (2004) Top down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. *Environmental Microbiology* 6, 733–744.

de Ruiter, P.C., Neutel, A.M. & Moore, J.C. (1998) Biodiversity in soil ecosystems: the role of energy flow and community stability. *Applied Soil Ecology* 10, 217–228.

Do, T. (2001) Characteristic of karst ecosystems of Vietnam and their vulnerability to human impact. *ACTA Geologica Sinica*, 75, 325–329.

FAO (1993) Forest Resources Assessment 1990. Tropical countries. *FAO Forestry Paper 112*, FAO, Rome.

Ferris, H., Bongers, T. & de Goede, R.G.M. (2001) A framework for soil food web diagnostics:extension of the nematode faunal analysis concept. *Applied Soil Ecology*, 18, 13–29.

Ferris, H. Venette, R. C. & Lau, S. S. (1997) Population energetics of bacterial-feeding nematodes: carbon and nitrogen budgets. *Soil Biology & Biochemistry*, 29, 1183–1194.

Filipjev, I.L. (1934) The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithsonian Miscellanean Collections*, 89, 1–63.

Flegg, J.J.M. (1967) Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology*, 60, 429–437.

Freckman, D.W. & Ettema, C.H. (1993) Assessing nematode communities in agroecosystems of varying human intervention. *Agriculture Ecosystem Environmental*, 45, 239–261.

Freckman, D. W. & Virginia, R. A. (1993) Extraction of nematodes from Dry Valley Antarctic soils. *Polar Biology*, 13, 483–487.

Gagarin, V.G. & Nguyen, V.T. (2003) Three new species of free-living nematodes from Vietnamese water bodies. *Zoologicheskii Zhurnal*, 82, 1393–1401.

Gagarin, V.G. & Nguyen, V.T. (2004) New species of the genera *Chronogaster* (Araeolaimida: Chronogasteridae) and *Dorylaimellus* (Dorylaimida: Belondiridae) from Vietnam (Nematoda). *Zoosystematica Rossica*, 12, 145–149.

Gagarin, V.G. & Nguyen, V.T. (2005) Three new species of free-living nematodes from freshwater bodies of north Vietnam. *International Journal of Nematology*, 15, 110–116.

Gagarin, V.G. & Nguyen, T.T. (2008a) Free-living nematodes from the Red River Delta, Vietnam. *Inland Waters Biology*, 1, 12-15.

Gagarin, V.G. & Nguyen, T.T. (2008b) Free-living nematodes from the Chu River, northern Vietnam, *Inland Waters Biology*, 1, 16–20.

Giller, K.E., Beare, M.H., Lavelle, P., Izac, A.M.N. & Swift, M.J. (1997) Agricultural intensification, soil biodiversity and agroecosystem function. *Applied Soil Ecology*, 6, 3–16.

Giller, P.S. (1996). The diversity of soil communities, the “poor man’s tropical rainforest”. *Biodiversity and Conservation*, 5, 135–168.

Grainger A. (2010) Uncertainty in the construction of global knowledge of tropical forests. *Progress in Physical Geography*, 34, 811–844.

Groombridge, B. (1992). *Global biodiversity: status of the Earth's living resources*. London, UK, Chapman & Hall.

Hansen, M.C. & DeFries, R.S. (2004) Detecting long-term global forest change using continuous fields of tree-cover maps from 8-km advanced very high resolution radiometer (AVHRR) data for the years 1982–99. *Ecosystems*, 7, 695–716.

Heyns, J. (1965) On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimoid nematodes. *Entomology Memoirs, Department of Agricultural Technical Services, Republic of South Africa*, 10: 1–51.

Holterman, M., Rybarczyk, K., van den Essen, S., van Megen, H., Mooyman, P., Peña-Santiago, R., Bongers, T., Bakker, J. & Helder, J. (2008) A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources*, 8, 23–34.

Holtkamp, R., Kardol, P., van der Wal, A., van der Putten, W.H., de Ruiter, P.C. & Dekker, S.C. 2008 Soil food web structure during ecosystem development after land abandonment. *Applied soil ecology*, 39, 23–34

Huelsenbeck, J.P. & Ronquist, F. (2001). MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.

Hugot, J. P., Baujard, P. & Morand, S. (2001) Biodiversity in helminthes and nematodes as a filed of study: an overview. *Nematology*, 3, 199–208.

Jairajpuri, M.S. & Ahmad, W. (1992) *Dorylaimida. Free-living, Predaceous and Plant-parasitic Nematodes*. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, India, 458 pp.

Jairajpuri, M.S. & Baqri, Q.H. (1966) *Sectonema procta* n sp, and *Pungentus angulatus* n sp, two new soil-inhabiting nematodes. *Nematologica*, 12, 396–402.

Jamieson, N.L., Cuc, L.T. & Rambo, T.A. (1998) The development crisis in Vietnam's mountains. *East-West Center special reports*, 6, 1–32.

Kim K.C. & Byrne L.B. (2006) Biodiversity loss and the taxonomic bottleneck: emerging biodiversity science. *Ecological Research*, 21, 794–810.

Korthalas, G.W., van de Ende, A., van Megen, H., Lexmond, T.M., Kammenga, J.E. & Bongers, T. (1996) Short-term effects of cadmium, copper, nickel, and zinc on soil nematodes from different feeding and life-history strategy groups. *Applied Soil Ecology*, 4, 107–117.

Lawton, J.H., Bignell, D.E., Bloemers, G.F., Eggleton, P. & Hodda, M.E. (1996) Carbon flux and diversity of nematodes and termites in Cameroon forest soils. *Biodiversity Conservation*, 5, 261–273

Lee, K.E. & Pankhurst, C.E. (1992) Soil organisms and sustainable productivity. Aust. *Journal Soil Respiration*, 30, 855–892.

Loof, P.A.A. & Coomans, A. (1970) On the development and location of the oesophageal gland nuclei in Dorylaimina. *Proceedings of the IX International Nematology Symposium (Warsaw, Poland, 1967)*, 79–161.

Maraun, M., Schatz, H. & Scheu, S. (2007) Awesome or ordinary? Global diversity patterns of oribatid mites. *Ecography* 30, 209–216.

Marchant, R. & Nicholas, W. (1974) An energy budget for the free-living nematode Pelodera (Rhabditidae). *Oecologia*, 16, 237–252.

McSorley, R. (2012) Ecology of the dorylaimid omnivore genera *Aporcelaimellus*, *Eudorylaimus* and *Mesodorylaimus*. *Nematology*, 14, 645–663.

McSorley, R. & Frederick, J.J. (1996) Nematodes community structure in rows and between rows of a soybean field. *Fundamental Applied Nematology*, 19, 251-261.

McSorley, R. & Frederick, J.J. (2004) Effect of extraction method on perceived composition of the soil nematode community. *Applied Soil Ecology*, 27, 55–63.

McSorley, R. & Parrado, J.L. (1987) Nematode losses during centrifugal extraction from two soil types. Nematropica 17, 147–161.

McSorley, R. & Walter, D.E. (1991) Comparison of soil extraction methods for nematodes and microarthropods. *Agriculture, Ecosystems and Environment*, 34, 201–207.

Moens, T., Dos Santos, G.A.P., Thompson, F., Fonsêca-Genevois, V. & de Mesel, I. (2005) Do nematodes mucus secrections affect bacterial growth? *Aquatic Microbial Ecology* 40, 77–83.

Mulder, C., Boit A., Bonkowski, M., De Ruiter, P.C., Mancinelli, G., Van der Heijden, M.G.A., Van Wijnen, H.J., Vonk, J.A., Rutgers, M. & Guy, W. (2011) A belowground perspective on Dutch agroecosystems: How soil organisms interact to support ecosystem services. *In: Advances in Ecological Research. Academic Press*, 277–357.

Mulder, C., Den Hollander, H., Vonk, J., Rossberg, A., Jagers op Akkerhuis, G. & Yeates, G. (2009) Soil resource supply influences faunal size-specific distributions in natural food webs. *Naturwissenschaften*, 96, 813–826.

Neher, D.A., Peck, S.L., Rawlings, J.O. & Campbell, C.L. (1995) Measures of nematode community structure and sources of variability among and within fields. *Plant Soil*, 170, 167–181.

Neher, D.A., Wu, J., Barbercheck, M.E. & Anas, O. (2005) Ecosystem type affects interpretation of soil nematode community measures. *Applied Soil Ecology*, 30, 47–64.

Nguyen, N.T. (1994) Diversity of the Cuc Phuong flora primary forest of Vietnam*. Proc. Nat. Cent. Nat. Sci. Techn.* Vietnam 6, 77–82.

Nguyen, N.T. (1997) *The vegetation of Cuc Phuong National Park*. Sida, 17, 713–751.

Nguyen, T.A.D. (2011) Free-living terrestrial nematodes (Dorylaimida) from Cuc Phuong National Park, Vietnam. Master Thesis, University of Jaén, Jaén, Spain. 115pp.

Nguyen, T.H. (1998) The gymnosperms of Vietnam. In: Floristic characteristics and diversity of East Asian plants. *Proceeding of the First International Symposium on Flora Characters and diversity of E Asian Plants. Kumming, 1996. Bejing, Berlin etc., China Higher Ed. Press, Spinger Verlag*, 91–103.

Nguyen, V.T. (2007) [Fauna of Vietnam 22]. *Science and technics publishing house*. Hanoi, Vietnam. 458 pp. (In Vietnamese).

Nicholas, K.B., Nicholas Jr, H.B. & Deerfield II, D.W. (1997). GeneDoc: analysis and visualization of genetic variation. *EMBnet News* 4, 1–14.

Nylander, J.A.A. (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Oostenbrink, M. (1960) Estimating nematode populations by some selected methods. *Nematology*, 6, 85–102.

Overgaard Nielsen, C. (1949) Studies on the soil microfauna II. The soil inhabiting nematodes. *Natura Jutlandica*, 2, 1–131.

Pearse, A.S. (1942) *An introduction to parasitology*. Baltimore, 375 pp.

Peña-Santiago, R. (2006) *Dorylaimida Part I: Superfamilies Belondiroidea, Nygolaimoidea and Tylencholaimoidea. In: Eyualem-Abebe, E., Traunspurger, W. & Andrássy, I. (Eds.). Freshwater Nematodes: Ecology and Taxonomy, 326–391*. CABI Publishing. Wallingford, UK. 752 pp.

Peña-Santiago, R. & Álvarez-Ortega, S. (2014) Studies on the genus *Sectonema* Thorne, 1930 (Dorylaimida: Aporcelaimidae). Redescription of *S. ventrale*, the type species of the genus. *Nematology*, 16, 1097–1104.

Peña-Santiago, R., Abolafia, J. & Álvarez-Ortega, S. (2014) New proposal for a detailed description of the dorylaim spicule (Nematoda: Dorylaimida). *Nematology*, 16, 1091–1095.

Perz, S.G. (2007) Grand theory and context-specificity in the study forest dynamics: forest transition theory and other directions. *The Professional Geographer*, 59, 105–114.

Popovici, P. (1978) New nematodes species (Dorylaimoidea) from Romania. *Nematologica*, 24, 404–411.

Porazinska, D.L., Giblin-Davis, R.M., Esquivel, A., Powers, T.O., Sung, W. & Thomas, W.K. (2010) Ecometagenetics confirm high tropical rainforest nematode diversity. *Molecular Ecology*, 19, 5521–5530.

Porazinska, D.L., Giblin-Davis, R.M., Powers, T.O. & Thomas, W.K. (2012) Nematode spatial and ecological patterns from tropical and temperate rainforests. *PloS ONE,* 7, e44641.

Powers T.O., Neher D.A., Mullin P., Esquivel A., Giblin-Davis R.M., Kanzaki N., Stock S.P., Mora M.M. & Uribe-Lorio L. (2009) Tropical nematode diversity: vertical stratification of nematode communities in a Costa Rican humid lowland rainforest. *Molecular Ecology*, 18, 985–996.

Pradhan, G. & Dash, M. (1987) Distribution and population dynamics of soil nematodes in a tropical forest ecosystem from Sambalpur, India*. Proceedings of the Indian Academy of Sciences (Animal Sciences)* 96, 395–402.

Price, N.S. & Siddiqi, M.R (1994) Rainforest nematodes with particular reference to the Korup National Park, Cameroon. *Afro-Asian Journal of Nematology* 4, 117–128.

Procter, D.L.C. (1984) Towards a biogeography of free-living soil nematodes. I. Changing species richness, diversity and densities with changing latitude. *Journal of Biogeography* 11, 103–117.

Ronquist, F. & Huelsenbeck, J. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.

Sanchez-Moreno, S., Ferris, H., Young-Mathews, A., Culman, S.W. & Jackson, L.E. (2011) Abundance, diversity and connectance of soil food web channels along environmental gradients in an agricultural landscape. *Soil Biology and Biochemistry*, 43, 2374–2383.

Shearman, P., Bryan J. & Laurance, W.F. (2012) Are we approaching 'peak timber' in the tropics?. *Biological Conservation*, 151, 17–21.

Siddiqi, M. R. (1984) *Sectonema anisonchum* sp. n., *S. mucrodens* sp. n. and *S. truxum* sp. n. (Nematoda: Dorylaimida) from Colombian rain forest. *Indian Journal of Nematology*, 14, 84–88.

Siddiqi, M.R. (1995) Nematodes of Tropical Rainforests. 5. Seven New Genera and Forty Two New Species of Dorylaims. *Afro-Asian Journal of Nematology*, 5, 72–109.

Sohlenius, B. (1973) Structure and dynamics of populations of Rhabditis (Nematodes: Rhabditidae) from forest soil. *Pedobiologia*, 13, 368–375.

Sohlenius, B. & Boström, S. (1984) Colonization, population development and metabolic activity of nematodes in buried barley straw. *Pedobiologia*, 27, 67–78.

Sterling, E.J., Hurley, M.M. & Le, D.M. (2006) *Vietnam: A Natural History*. Yale University Press, New Haven, 448 pp.

Stinner, B.R. & Crossley, D.A., Jr. (1982) Nematodes in no-tillage agroecosystems. In: Freckman, D.W. (ed.) *Nematodes in Soil Ecosystems*. University of Texas Press, Austin, 14–28.

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455–474.

Sudhaus, W. (1981) Über die Sukzession von Nematoden in Kuhfladen. *Pedobiologia*, 21, 271–297.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.M., Ekschmitt, K., Kjoller, A., Loiseau, P. & Smith, P. (1998) Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. *Global Change Biology*, 4, 729–743.

Swofford, D.L. (2003) PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b 10. Sunderland, MA: Sinauer Associates.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.

Tetuna, M. & Ferris, H. (2004) Relationship between nematode life-history classification and sensitivity to stressor: ionic and osmotic effects of nitrogenous solutions. *Journal of Nematology,* 36, 85–94.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876–4882.

Thorne, G. (1930) Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. *Journal of Agricultural Research*, *USDA*, 41, 445–466.

Tordoff, A. W., Vu Van Dung, Le Van Cham, Tran Quang Ngoc & Dang Thang Long (2000) A rapid field survey of five sites in Bac Kan, Cao Bang and Quang Ninh provinces: a review of the Northern Indochina Subtropical Forests Ecoregion. Hanoi: BirdLife International Vietnam Programme and the Forest Inventory and Planning Institute. In English and Vietnamese.

Traunspurger, W., Bergtold, M. & Goedkoop, W. (1997) The effect of nematodes on bacterial activity and abundance in a freshwater sediment. *Oecologia*, 112, 118–122.

Tsalolikhin, S.J. (1976) Free-living nematodes as indicators of polluted freshwaters. In: Skarlato, O.A. (ed.) *Methods of Biological Analysis of Freshwaters*. Akademia Nauk, Leningrad, 118–122.

Twinn, D.C. (1974) Nematodes. In: Dickinson, C.H. and Pugh, G.J.F. (eds) *Biology of Plant Litter Decomposition* II. Academic Press, London, 421–465.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, 11, 1351–1363.

Verschoor, B.C., de Goede, R.G.M., de Hoop, J.-W. & de Vries, F.W. (2001) Seasonal dynamics and vertical distribution of plantfeeding nematode communities in grasslands. *Pedobiologia*, 45, 213-233

Vinciguerra, M. T. (2006) *Dorylaimida Part II: Superfamilies Dorylaimoidea. In: Eyualem-Abebe, Traunspurger, W. & Andrássy, I. (Eds.), Freshwater nematodes: Ecology and Taxonomy, 392-467.* CAB International. Wallingford, UK. 752 pp.

Vinciguerra, M.T.; Orselli, L. & Clausi, M. (2014) One new and two known species of *Aporcelinus* Andrássy, 2009 and a new species of *Coomansinema* Ahmad & Jairajpuri, 1989 (Nematoda: Dorylaimida). *Nemaology*, 16, 303–322.

Vu, T.T.T, Ciobanu, M., Abolafia, J. & Peña-Santiago, R. (2010) Two remarkable new species of the genus *Crassolabium* Yeates, 1967 from Vietnam (Nematoda: Dorylaimida: Qudsianematidae). *Journal of Natural History*, 44, 2049–2064.

Wardle D., Yeates G., Watson R. & Nicholson K. (1995) The detritus food-web and the diversity of soil fauna as indicators of disturbance regimes in agro-ecosystems. *Plant and Soil*, 170, 35–43.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629–1633.

Wasilewska, L. (1970) Nematodes of the sand dunes in the Kampinos Forest. I. Species structure. *Ekologia Polska,* 18, 429–443.

Wieser, W. (1956) Free living marine nematodes III. Aconolaimoidea and Monhysteroidea. *In Reports of the Lund University Chile Expedition*, 1948–1949, Lund, 1–15.

Williams, B.D., Schrank, B., Huynh, C., Shownkeen, R. & Waterston, R.H. (1992) A genetic mapping system in Caenorhabditis elegans based on polymorphic sequence tagged sites. *Genetics*, 131, 609–624.

Yeates G.W. & Bongers T. (1999) Nematode diversity in agroecosystems. *Agriculture, Ecosystems & Environment*, 74, 113–135.

Yeates, G.W. & Coleman, D.C. (1982) Role of nematodes in decomposition. In: Freckman, D.W. (ed.) *Nematodes in Soil Ecosystems*. University of Texas Press, Austin, 55–81.

Yeates, G.W. (1979) Soil nematodes in terrestrial ecosystems. *Journal of Nematology*, 11, 213–229.

Yeates, G. W, Bardgett, R.D., Cook, R., Hobbs, P.J., Bowling, P.J. & Potter, J.F. (1997) Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes.*Journal Applied Ecology*, 34, 453–470.

Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W. & Georgieva, S.S. (1993) Feeding habits in nematode families and genera: an outline for soil ecologists. *Journal of Nematology*, 25, 315–331.

Yeates, G.W., Ferris, H., Moens, T. & Van der Putten, W.H. (2009) The role of nematodes in ecosystems. In: Wilson, M.J. & Kakouli-Duarte, T. (Eds). *Nematodes as environmental indicators*. Wallingford, UK, CAB International, pp. 1–44.

Yeates, G.W., Wardle, D.A., Watson, R.N. (1999) Responses of soil nematode populations, community structure, diversity and tem poral variability to agricultural intensification over a seven-year period. *Soil Biology & Biochemistry*, 31, 1721–1733.

Yoder, M., De Ley, I. T., King, I. W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L. & De Ley, P. (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology*, 8, 367–376.

Zaborski, Ed. (2014) Soil nematodes in organic farming systems. University of Illinois. *eOrganic article*. eOrganic4495

Zhang, M., Liang, W-J. & Zhang, X-K. (2012) Soil nematode abundance and diversity in different forest types at Changbai Mountain, China. *Zoological Studies*, 51, 619–626.

Zullini, A. & Peretti, E. (1986) Lead pollution and moss-inhabiting nematodes of an industrial area. *Water, Air, Soil Pollution*, 27, 403–410.

Zullini, A. (1976) Nematodes as indicators of river pollution. *Nematologia Mediterranea*, 4, 13–22.

6. Appendix

APPENDIX 1. Mean relative abundance of nematodes per 100gr in soils at different land use intensity.

	Nematode genera	Functional group	C-p[a] Value	P-P Value	Land use type			
					Primary forest	Secondary forest	Slash and burn	Intensive agricuture
	Order Alaimida Siddiqi, 1983							
	Family Alaimidae Micoletzky, 1922							
1	*Alaimus*	Bacterivores	4	0	54.3	25.4	27.0	24.0
	Family Amphidelidae Andrássy, 2002							
2	*Amphidelus*	Bacterivores	4	0	7.3	0.8	5.5	9.2
	Order Aphelenchida Siddiqi, 1980							
	Family Aphelenchidae Fuchs, 1937							
3	*Aphelenchus*	Fungivores	2	0	0	0	0.4	32.7
	Family Aphelenchoididae Skarbilovich, 1947							
4	*Aphelenchoides*	Fungivores	2	0	25.7	32.6	12.0	43.1
	Order Araeolaimida De Coninck & Schuurmans Stekhoven, 1933							
	Family Aphanolaimidae Chitwood, 1936							
5	*Aphanolaimus*	Bacterivores	3	0	0	0.1	0.3	1.5
	Family Plectidae Örley, 1880							
6	*Anaplectus*	Bacterivores	2	0	0	0	6.4	1.2
7	*Plectus*	Bacterivores	2	0	3.2	0	0.1	0.9
8	*Tylocephalus*	Bacterivores	2	0	0	0	0.1	0
9	*Wilsonema*	Bacterivores	2	0	0	0	2.0	0
	Order Chromadorida Chitwood, 1933							
	Family Chromadoridae Filipjev, 1917							
10	*Chromadorina*	Bacterivores	3	0	3.3	0	3.5	0
11	*Punctodora*	Omnivores	4	0	0	0	2.7	0
	Order Diphtherophorida Loof, 1991							
	Family Trichodoridae Thorne, 1935							
12	*Trichodorus*	Herbivores-ectoparasites	0	4	0	3.5	3.2	0.4
	Order Dorylaimida Pearse, 1942							
	Family Actinolaimidae Thorne, 1939							
13	*Paractinolaimus*	Predators	5	0	54.4	19.0	31.0	2.2
14	*Actinolaimus*	Omnivores	4	0	0.4	0.4	0.6	0
	Family Aporcelaimidae Heyns, 1965							
15	*Aporcelaimellus*	Omnivores	5	0	58.5	19.1	25	24.9
16	*Aporcelaimoides*	Omnivores	5	0	0	0.4	0	2.6
17	*Aporcelaimus*	Omnivores	5	0	1.9	1.8	1.8	9.3
18	*Aporcelinus*	Omnivores	5	0	2.8	0	0	0
19	*Sectonema*	Omnivores	5	0	7.0	10.6	7.5	1.9

	Family Belondiridae Thorne, 1939							
20	*Axonchium*	Herbivores-ectoparasites	0	5	42.1	1.8	5.8	8.0
21	*Belondira*	Herbivores-ectoparasites	0	5	39.7	4.3	9.9	26.3
22	*Metaxonchium*	Herbivores-ectoparasites	0	5	25.5	0.4	7.6	0.5
23	*Oxybelondira*	Herbivores-ectoparasites	0	5	12.1	7.4	6.7	4.0
24	*Oxydirus*	Predators	5	0	0	0.6	0.7	3.1
	Family Dorylaimidae de Man, 1876							
25	*Dorylaimus*	Omnivores	4	0	60.8	45.6	80.9	26.2
26	*Laimydorus*	Omnivores	4	0	0	0	0	0.1
27	*Drepanodorylaimus*	Omnivores	4	0	22.6	4.1	8.2	0.2
28	*Mesodorylaimus*	Omnivores	4	0	70.8	102	14.2	40.7
29	*Prodorylaimium*	Omnivores	4	0	33.9	3.8	7.1	0.8
30	*Prodorylaimus*	Omnivores	4	0	12.5	2.7	2.8	0.5
	Family Leptonchidae Thorne, 1945							
31	*Funaria*	Fungivores	4	0	1.8	0	0.7	0
32	*Leptonchus*	Fungivores	4	0	0	2.3	1.7	1.3
33	*Proleptonchus*	Fungivores	4	0	0	1.2	0	0
34	*Tyleptus*	Omnivores	4	0	6.9	3.0	0.9	0
	Family Longidoridae Thorne, 1935							
35	*Longidorus*	Herbivores-ectoparasites	0	5	5.7	26.9	7.6	9.8
36	*Paralongidorus*	Herbivores-ectoparasites	0	5	0	2.9	0	0.4
37	*Xiphinema*	Herbivores-ectoparasites	0	5	61.0	43.0	63.2	8.3
	Family Mydonomidae Thorne, 1964							
38	*Dorylaimoides*	Fungivores	4	0	17.5	9.7	0.1	0.7
	Family Nordiidae Jarajpuri & Siddiqi, 1964							
39	*Enchodorus*	Omnivores	4	0	18.8	9.3	2.0	0
40	*Longidorella*	Herbivores-ectoparasites	0	4	4.0	2.9	1.2	0.4
41	*Oriverutus*	Omnivores	4	0	80.6	86.2	19.3	12.5
42	*Rhyssocolpus*	Omnivores	4	0	0	0.3	0	0.2
	Family Qudsianematidae Jarajpuri, 1965							
43	*Allodorylaimus*	Omnivores	4	0	9.6	4.0	6.5	6.2
44	*Crassolabium*	Omnivores	4	0	89.9	28.4	47.1	31.25
45	*Discolaimium*	Predators	5	0	0	0	0.6	0.4
46	*Discolaimoides*	Predators	5	0	0	0	0	1.3
47	*Discolaimus*	Predators	5	0	0	0.3	0	0.6
48	*Labronema*	Omnivores	4	0	20.1	46.3	27.0	1.52
49	*Labronemella*	Omnivores	4	0	2.9	2.5	1.6	0
50	*Epidorylaimus*	Omnivores	4	0	4.4	0.6	3.2	0
51	*Eudorylaimus*	Omnivores	4	0	265.5	61.68	117.4	145.9
52	*Microdorylaimus*	Omnivores	3	0	0	0	0	1.2

53	*Mylodiscus*	Omnivores	4	0	0	0.7	0	0.4
	Family Thornenematidae Siddiqi, 1969							
54	*Coomansinema*	Omnivores	4	0	9.8	0	1.2	0
55	*Opisthodorylaimus*	Omnivores	5	0	10.7	30.5	85.7	99.6
	Family Tylencholaimidae Filipjev, 1934							
56	*Tylencholaimus*	Fungivores	4	0	22.2	23.5	6.1	0
	Family Tylencholaimellidae Jairajpuri, 1964							
57	*Tylencholaimellus*	Fungivores	4	0	0	3.6	3.6	1.3
	Order Nygolaimina Ahmad & Jairajpuri, 1979							
	Family Nygolaimidae Thorne, 1935							
58	*Laevides*	Predators	5	0	2.4	0.9	0.3	0.2
59	*Nygolaimus*	Predators	5	0	0.5	0	0.6	0
60	*Nygolaimellus*	Predators	5	0	3.3	0.1	1.2	1.9
	Order Enoplida Filipjev, 1929							
	Family Ironidae de Man, 1876							
61	*Ironus*	Predators	4	0	94.8	13.6	48.7	30.6
	Family Prismatolaimidae Micoletzky, 1922							
62	*Prismatolaimus*	Bacterivores	3	0	0	0	0.3	0.4
	Family Tobrilidae De Coninck, 1965							
63	*Tobrilus*	Predators	3	0	23.2	2.6	9.2	5.4
	Family Tripylidae de Man, 1867							
64	*Trypila*	Predators	3	0	1.2	1.2	1.7	0.8
	Order Monhysterida De Coninck & Schuurmans Stekhoven, 1933							
	Family Monhysteridae de Man, 1876							
65	*Geomonhystera*	Bacterivores	2	0	0	0	0.6	0.6
	Family Xyalidae Chitwood, 1951							
66	*Daptonema*	Bacterivores	2	0	5.3	3.0	1.6	0.2
67	*Theristus*	Bacterivores	2	0	3.0	0.9	2.7	2.2
	Order Mononchida Jairajpuri, 1969							
	Family Anatonchidae Jairajpuri, 1939							
68	*Iotonchus*	Predators	4	0	36.7	186.5	61.6	26.8
	Family Mononchidae Filipjev, 1934							
69	*Actus*	Predators	4	0	28.9	0.5	0	8.3
70	*Clarkus*	Predators	4	0	8.4	15.1	1.0	0
71	*Cobbonchus*	Predators	4	0	0	0	1.2	0.4
72	*Coomansus*	Predators	4	0	43.1	0.7	2.4	0.5
73	*Judonchulus*	Predators	4	0	0	0	5.96	0
74	*Mononchus*	Predators	4	0	7.2	2.9	1.1	3.0
75	*Prionchulus*	Predators	4	0	3.1	1.3	3.2	0
	Family Mylonchuilidae Jairajpuri, 1969							
76	*Mylonchulus*	Predators	4	0	43.5	177.9	16.9	15.9
	Order Rhabditida Chitwood, 1933							
	Family Bicirronematidae							
77	*Bicirronema*	Bacterivores	1	0	0.4	1.6	0	0

	Family Cephalobidae Filipjev, 1934							
78	*Acrobeles*	Bacterivores	2	0	0	0	0	1.5
79	*Acrobeloides*	Bacterivores	2	0	0	0	0	6.1
80	*Acrolobus*	Bacterivores	2	0	0	0	0	2.9
81	*Cephalobus*	Bacterivores	2	0	0	0	0.3	0
82	*Chiloplacus*	Bacterivores	2	0	0	0	0	1.2
83	*Eucephalobus*	Bacterivores	2	0	1.2	0.1	2.3	32.
84	*Pseudacrobeles*	Bacterivores	2	0	0	0	0.4	5.7
85	*Zeldia*	Bacterivores	2	0	0	0	0	1.7
	Family Diplogastridae Micoletzky, 1922							
86	*Diplogaster*	Bacterivores	1	0	0.5	0	0.1	0
	Family Mesorhbditidae Andrássy, 1976							
87	*Cruznema*	Omnivores	4	0	8.8	0	0	0
88	*Mesorhabditis*	Bacterivores	1	0	20.8	6.9	4.0	19.2
	Family Panagrolaimidae Thorne, 1937							
89	*Panagrolaimus*	Bacterivores	1	0	1.6	1.3	4.1	11.7
	Family Pelodera Andrássy, 1976							
90	*Caenorhabditis*	Bacterivores	1	0	5.9	0.3	6.2	33.6
	Order Tylenchida Thorne 1949							
	Family Criconematidae Taylor, 1936							
91	*Criconema*	Herbivores-ectoparasites	0	3	1.1	12.5	0	0.4
92	*Discocriconemella*	Herbivores-ectoparasites	0	3	0	0.8	20.1	4.0
93	*Hemicriconemoides*	Herbivores-ectoparasites	0	3	0	0	15.0	23.8
	Family Hoplolaimidae Filipjev, 1934							
94	*Helicotylenchus*	Herbivores-semi endo parasites	0	3	0	15.6	11.5	39.5
95	*Hoplolaimus*	Herbivores-semi endo parasites	0	3	0	0	0	17.8
96	*Rotylenchus*	Herbivores-semi endo parasites	0	3	0	2.0	0	3.7
97	*Scutellonema*	Herbivores-semi endo parasites	0	3	0	0	12.7	0.5
	Family Psilenchidae Paramonov, 1967							
98	*Psilenchus*	Herbivores-epidermal/root hair feeder	0	2	0	0	0.8	6.2
	Family Pratylenchidae Thorne, 1949							
99	*Hirschmanniella*	Herbivores-migratory endo parasites	0	3	0	0	0	4.1
100	*Pratylenchus*	Herbivores-migratory	0	3	0	0	0	0.2

		endo parasites						
	Family Steinernematidae							
101	*Steinernema*		1	0	0	0	2.5	0.9
	Family Telotylenchidae Siddiqi, 1960							
102	*Amplimerlinius*	Herbivores-ectoparasites	0	3	0	0	1.2	1.4
103	*Tylenchorhynchus*	Herbivores-ectoparasites	0	3	0	4.3	90.1	26.0
	Family Tylenchidae Oerley, 1880							
104	*Filenchus*	Fungivores	2	0	0	3.7	1.9	67.3
105	*Tylenchus*	Herbivores-epidermal/root hair feeder	0	2	17.6	4.0	9.2	38.

[a] Colonizer-persister scale 1-5 where cp 1 are colonizers characterized by short generation time and cp 5 are persisters characterized by long generation time (Bonger, 1990).

7. Acknowledgements

This thesis could not have been completed without the help, encouragement and support from a number of people, to whom I would like to send my sincere gratitude.

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Con xin gửi lời cảm ơn tới bố mẹ người đã sinh ra con, nuôi dậy con khôn lớn và trưởng thành như ngày hôm nay. Em gái Thùy Dương, người bạn thân luôn luôn lắng nghe, động viên chị. Mẹ Dung, người đặc biệt chia sẻ những vất vả khó khăn chăm sóc bạn Dâu Tây khi con vắng nhà. Con xin cảm ơn bạn bè và người thân trong gia đình hai bên nội ngoại đã luôn quan tâm động viên để con hoàn thành luận văn này!!!

8. Declaration of Contribution as Author and Co-Author

I am the first author and main contributor in six publications submitted for my dissertation including:

- Conducting field surveys
- Taking the soil samples in the field, nematode extraction and description of environmental parameters
- Morphological examination and taxonomy of nematodes
- Data handling and processing
- Conducting statistical analyses
- Interpretation of data
- Writing manuscripts
- Submission process and correspondence together with supervisors to editors and/or reviewers of scientific journals

In addition, I am a co-author in three publications, where I contributed the following contents:

- Conducting field surveys
- Collecting the soil samples from the field trip, nematode extraction and description of environmental parameters
- Extracting and mounting the nematodes on permanent slide for the identification and documentation
- Identifying the nematodes to species level
- Taking the measurements of all the specimens
- Providing morphological data tables and figures
- Revising the manuscripts during its preparation and the reviewing process.

Dissertation Title: Community structure and ecological niches of free-living terrestrial nematodes in north-eastern Vietnam.

The author's contribution is categorized in three levels:

A. has contributed to the collaboration (0-33%);

B. has contributed substantially (34-66%);

C. has to a high degree carried out the work independently (67-100%)

Publication 1

1. **Nguyen. T.A.D,** Vu. T.T.T, Bonkowski. M. & Peña-Santiago. R**.** (2014). New data of three rare belondirid species (Nematoda, Dorylaimida, Belondiridae) from Vietnam, with the first record and description of the male of *Oxybelondira paraperplexa* Ahmad & Jairajpuri, 1979. *Biodiversity Data Journal* 2. e1156. Doi: 10.3897/BDJ. 2. e1156.

Declaration in each element	**A, B, or C**
1. Formulating the scientific hypotheses based on theoretical assumptions to be clarified, including formulation of the question to be answered through analytical work and research plans.	**C**
2. Planning of experiments and analyses, design of the experimental methods in a way that the hypotheses can be expected to be answered.	**C**
3. Analytical work in the experimental studies and investigations.	**C**
4. Presentation, interpretation and discussion of the results.	**C**

Publication 2

2. Álvarez-Ortega. S**,** **Nguyen. T.A.D,** Abolafia. J, Vu. T.T.T & Peña-Santiago. R**.** (2015). Three new species of the genus *Aporcelaimoides* Heyns, 1965 from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae), with an updated taxonomy of the genus. *ZooKeys* 516:1-26. Doi: 10.3897/zookeys.516.10087

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3. Analytical work in the experimental studies and investigations.	**B**
4. Presentation, interpretation and discussion of the results.	**A**

Publication 3

3. **NGUYEN. T.A.D,** ABOLAFIA. J, BONKOWSKI. M, PEÑA-SANTIAGO. R & ÁLVAREZ-ORTEGA. S (2015). Two atypical new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. *European Journal of taxonomy* 171: 1-20. Doi: 10.5852/ejt.2016.171

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4. Presentation, interpretation and discussion of the results.	**C**

Publication 4

4. ÁLVAREZ-ORTEGA. S , **NGUYEN. T.A.D,** ABOLAFIA. J, VU. T.T.T , BONKOWSKI. M, PEÑA-SANTIAGO. R (2015)**.** Three new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam. *Nematology* 18(2016): 517-536. Doi: 10.1163/15685411-00002974

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3. Analytical work in the experimental studies and investigations.	**C**
4. Presentation, interpretation and discussion of the results.	**B**

Publication 5

5. **NGUYEN. T.A.D,** BONKOWSKI. M, PEÑA-SANTIAGO. R and ABOLAFIA. J. (2016). Redescription of *Cephalobus topali* Andrássy, 1970 (Rhabditida, Cephalobidae) from Vietnam and transfer to *Acrobeloides* (Cobb, 1924) Thorne, 1937. *Zootaxa* 4092 (4): 593-600. Doi: 10.11646/zootaxa.4092.4.9

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2. Planning of experiments and analyses, design of the experimental methods in a way that the hypotheses can be expected to be answered.	**C**
3 Analytical work in the experimental studies and investigations.	**C**
4. Presentation, interpretation and discussion of the results.	**C**

Publication 6

6. **NGUYEN. T.A.D,** BONKOWSKI. M, ABOLAFIA. J, and PEÑA-SANTIAGO. R (2016). Two new species of the genus *Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida) from Vietnam. *Zootaxa* 4103 (6): 550-560. Doi: 10.11646/zootaxa.4103.6.5

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3. Analytical work in the experimental studies and investigations.	**C**
4. Presentation, interpretation and discussion of the results.	**C**

Publication 7

7. Álvarez-Ortega. S, **Nguyen. T.A.D,** Abolafia. J, Bonkowski. M, Peña-Santiago. R (2016). *Sectonema caobangense* sp. n. from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae). *Journal of Nematogy* 48(2): 95-103.

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1. Formulating the scientific hypotheses based on theoretical assumptions to be clarified, including formulation of the question to be answered through analytical work and research plans.	**B**
2. Planning of experiments and analyses, design of the experimental methods in a way that the hypotheses can be expected to be answered.	**C**
3. Analytical work in the experimental studies and investigations.	**B**
4. Presentation, interpretation and discussion of the results.	**B**

Publication 8

8. **Nguyen. T.A.D,** Bonkowski. M, Abolafia. J, and Peña-Santiago. R (2016). A third new species *of Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam, with the first SEM study of a representative of the genus. *Journal of Nematogy* 48(2): 104-108.

Declaration in each element	**A, B, or C**
1. Formulating the scientific hypotheses based on theoretical assumptions to be clarified, including formulation of the question to be answered through analytical work and research plans.	**C**
2. Planning of experiments and analyses, design of the experimental methods in a way that the questions asked under point 1 can be expected to be answered.	**C**
3 Analytical works in the experimental studies and investigations.	**C**
4. Presentation, interpretation and discussion of the results.	**C**

Publication 9

9. **Nguyen. T.A.D,** Abolafia. J, Peña-Santiago. R & Bonkowski. M. (2016). Shifts in nematode community composition and function due to land-use intensification in tropical soils of Vietnam (in preparation).

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2. Planning of experiments and analyses, design of the experimental methods in a way that the hypotheses can be expected to be answered.	**C**
3. Analytical work in the experimental studies and investigations.	**C**
4. Presentation, interpretation and discussion of the results.	**C**

Dissertation author's signature

Date: Signature

Köln, 14.11.2016 ___________________________

(Thi Anh Duong Nguyen)

9. Erklärung

Erklärung (gemäß § 4 Abs 1 Punkt 9 der Prüfungsordnung). Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit einschließlich Tabellen, Karten und Abbildungen , die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie abgesehen von unten angegebenen Teilpublikationen − noch nicht veröffentlicht worden ist, sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Michael Bonkowski betreut worden.

Köln, 14.11.2016 ___________________________

(Thi Anh Duong Nguyen)

Statutory Declaration and Statement

I declare that I have authored this doctoral thesis independently, that I have not used other than the declared sources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

My dissertation was supervised by Prof. Dr. Michael Bonkowski University of Cologne and Prof. Dr. Reyes Peña-Santiago University of Jaen, Spain.

Publications:

1. **NGUYEN. T.A.D,** VU. T.T.T, BONKOWSKI. M. & PEÑA-SANTIAGO. R**.** (2014). New data of three rare belondirid species (Nematoda, Dorylaimida, Belondiridae) from Vietnam, with the first record and description of the male of *Oxybelondira* paraperplexa Ahmad & Jairajpuri, 1979. *Biodiversity Data Journal*. Doi: 10.3897/BDJ. 2. e1156.
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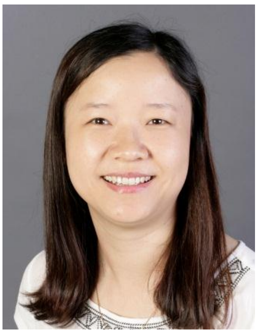
7. ÁLVAREZ-ORTEGA. S, **NGUYEN. T.A.D,** ABOLAFIA. J, BONKOWSKI. M, PEÑA-SANTIAGO. R (2016)**.** *Sectonema caobangense* sp. n from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae). *Journal of Nematogy* 48(2): 95-103.
8. **NGUYEN. T.A.D,** BONKOWSKI. M, ABOLAFIA. J, PEÑA-SANTIAGO. R (2016). A third new species *of Aporcelinus* Andrássy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from Vietnam, with the first SEM study of a representative of the genus. *Journal of Nematogy* 48(2): 104-108.
9. **NGUYEN. T.A.D,** ABOLAFIA. J, PEÑA-SANTIAGO. R, BONKOWSKI. M. (Submitted). Shifts in nematode community composition and function due to land-use intensification in tropical soils of Vietnam. *Soil Biology and Biochemistry*.

Köln, 14.11.2016

(Thi Anh Duong Nguyen)

10. Curriculum Vitae



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Education

10/2013 **03/2017**	**PhD student at University of Cologne** Department of Terrestrial Ecology, Institute of Zoology Faculty of Mathematics and Natural Sciences, University of Cologne, Germany
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09/2002 **09/2006**	**Bachelor of Science** Faculty of Biology, University of Natural Sciences, Ho Chi Minh, Vietnam Thesis: "Zooplankton in Tram Chim National Park, Dong Thap Province, Vietnam"
09/1998 **09/2001**	**Diploma of secondary education** Yen Vien Secondary School, Hanoi, Vietnam

Professional Experience

1/10/2013 **Up to now**	**PhD student at Cologne University** Department of Terrestrial Ecology, Institute of Zoology Faculty of Mathematics and Natural Sciences, University of Cologne, Germany Supported by DAAD scholarship ♦ Conducting PhD project the soil nematode fauna in limestone karst areas of Cao Bang/Lang Son in the mainland of northeastern Vietnam and of Cat Ba island in the Gulf of Tonkin.

15/09/2011 31/5/2013	**Researcher, Department of Nematology – Institute of Ecology and Biological Resources (IEBR), Viet Nam Academy of Science and Technology (VAST)** ♦ Using free-living terrestrial nematodes as bio-indicators and their relationship to environmental factors such as physical soil characteristics, chemical elements and human impact. ♦ Applying the non-pathogenic fungi such as *Fusarium oxyporum* against the root-knot nematode *Meloidogyne incognita* on tomato.
01/09/2007 31/08/2009	**Researcher, Department of Nematology – Institute of Ecology and Biological Resources (IEBR), Viet Nam Academy of Science and Technology (VAST)** ♦ Taxonomy of plant parasitic nematodes and studies on the ability of beneficial micro-organisms for bio-enhancement of crop plants against parasitic nematodes. ♦ Taxonomy of free-living nematodes in soils with focus on Mononchida and Dorylaimida and study on use them as bio-indicators for assessment of soil qualities.

Scientific Awards; Fellowship & Grants

6/2013 - 3/2017	Research Grants for Doctoral Candidates and Young Academic Scientists funded by the German Academic Exchange Service (DAAD)
2014 2015	Field work in Vietnam funded by German Academic Exchange Service (DAAD), Department of Terrestrial Ecology, University of Cologne and Idea Wild (United States of America)
9/2009 6/2011	European Master of Science in Nematology funded by Erasmus Mundus program from European Union

Scientific Associations

1/2016	Member of European Society of Nematologist (ESN)
1/2016	Member of SON (Society of Nematologist) from America and

International workshops and conferences

28/8/2016 2/9/2016	The 32 Symposium of European Society of Nematologist (ESN), 28/8-2/9/2016, Braga, Portugal Oral Presentation 1. Effects of land-use intensity on nematode communities of tropical soils in Vietnam.

22/8/2016
26/8/2016

XVII International Colloquium on Soil Zoology and XIV International Colloquium on Apterygota, Soil biodiversity for future Earth, Nara, Japan, 2016

Poster presentation

1. Species diversity of free-living nematodes in tropical soils of Vietnam, exemplified in the family Aporcelaimidae (Nematoda).
2. Effects of land-use intensity on the community composition of nematodes from Cao Bang Province in Vietnam.

17/7/2016
21/7/2016

Join meeting of Society of Nematologists (SON) and Organization of Nematologists of Tropical America (ONTA), Montreal, Canada

Poster presentation

1. Effects of land-use intensity on the community composition of nematodes from Cao Bang Province in Vietnam.

15/06/2016

Colloquium in Biocentrum

Oral Presentation:

1. Community structure and ecological niches of free-living terrestrial nematodes in north-eastern Vietnam.

15/5/2016
17/5/2016

PINC alumni Seminar days in University of Gent, Belgium

Oral Presentation:

1. Community structure and ecological niches of free-living terrestrial nematodes in north-eastern Vietnam.

2/12/2014
5/12/2014

The First Global Soil Biodiversity Conference: Assessing soil biodiversity and its role for ecosystem services, 2-5 December 2014, Dijon, France.

Poster presentation

1. Species diversity of free-living nematodes in tropical soils of Vietnam, exemplified in the order Dorylaimida (Duong Nguyen, Michael Bonkowski and Reyes Peña-Santiago).
2. Effects of land-use intensity on nematode communities of tropical soils in Vietnam (Duong Nguyen and Michael Bonkowski).

11/7/2010

The 14th International Meiofauna Conference, Ghent, Belgium

10/2008

LIPI – NaGISA Western Pacific Conference (meiobenthos in Western Pacific). LIPI – Jakarta – Indonesia

19/03/2008
25/03/2008

The 9th NaGISA taxonomy Conference on Nematoda. Identification of Marine nematodes. Seto Marine Biological Laboratory Shirahama, Japan