UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

ABORDAGEM TAXONÔMICA E PROTÉICA DE NEMATODA DE VIDA LIVRE

BETÂNIA CRISTINA GUILHERME

Recife

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Co-orientadores: Prof^{o} Dr^{o} André Morgado Esteves

Dr^{a} Elba Verônica Matoso Maciel de Carvalho

Colaboradores: Prof^{a} Dr^{a} Verônica da Fonsêca-Genevois

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BETÂNIA CRISTINA GUILHERME

ABORDAGEM TAXONÔMICA E PROTÉICA DE NEMATODA DE VIDA LIVRE

BANCA EXAMINADORA

TITULARES:

Profª Drª Maria Tereza dos Santos Correia (UFPE)

Prof. Dr. Francisco José Victor de Castro (UFCG)

Profª Drª Patrícia Maria Guedes Paiva (UFPE)

Profª Drª Maria Eduarda Lacerda de Larrazábal da Silva (UFPE)

Profª Drª Luciana Iannuzzi (UFPE)

SUPLENTE

Profª Drª Vera Lúcia de Menezes Lima(UFPE)

RECIFE

MARÇO, 2010

“*O que importa na vida não é o ponto de partida, mas a caminhada. Caminhando e semeando, no fim terás o que colher”. Cora Coralina.*

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RESUMO

Os Nematoda marinhos estão entre os grupos mais comuns, dominantes e diversos metazoários bentônicos. Normalmente, representam 70–90% de abundância, onde apresentam papéis ecológicos fundamentais. Estimativas de diversidade de Nematoda marinho são em número de milhões de espécies, da qual só uma pequena fração foi descrito. Estudos taxonômicos da família Thoracostomopsidae Filipjev, 1927 são raros, logo faz necessário um estudo que venha contribuir para o conhecimento dos seus representantes no Brasil. Este estudo teve como objetivos: (1) realizar um levantamento sobre o "estado da arte" da Família Thoracostomopsidae com base na bibliografia existente; (2) Descrever as novas espécies desta família obtidas em amostras coletadas nas Bacias de Campos e Potiguar e (3) levantar a composição protéica da nematofauna de dois ambientes costeiros. Na Bacia de Campos foram coletadas amostras de sedimento em nov/dez de 2002 (OCEANPROF I) e jun/jul de 2003 (OCEANPROF II) a bordo do navio Astro Garoupa. Um total de 43 estações ao longo de 9 transectos em profundidades de 750 m, 1050 m, 1350 m, 1650 m e 1950 m. Na Bacia Potiguar as amostras foram coletadas em duas campanhas (em 2004, com 69 estações, sendo 43 na malha de caracterização ambiental e 26 na malha de monitoramento ambiental dos emissários submarinos do pólo industrial de Guamaré; Em 2008, 36 estações de coleta, sendo 26 estações dos emissários submarinos do pólo industrial de Guamaré e 10 localizadas a 50m, 200m, 500m, 1000m e 2000m de distância dos emissários). As amostras foram fixadas com formaldeído (4%) salino e acondicionadas em potes plásticos. Em laboratório as amostras foram elutriadas para retirada dos Nematoda. Logo após foram confeccionadas lâminas permanentes para identificação genérica e específica, a partir de determinação dos dados de morfometria, através de literatura específica. Para o levantamento protéico foram coletados os Nematoda da praia de Maracaípe e Estuário do Pina. As amostras biosedimentológicas foram coletadas com um tubo PVC de 3,7 cm de diâmetro interno, sendo este inserido nos sedimentos até 10 cm de profundidade, no mediolitoral inferior, em seguida fixadas utilizando-se diferentes tratamentos (potes com formol, formol tamponado com bórax e sem formol) sendo todas mantidas resfriadas. O sedimento foi elutriado para análise protéica, sendo retirados 200 Nematoda de cada tratamento, nos quais, foram sonicados e centrifugados para análise protéica usando método BCA (Protein Assay Kit). Através do levantamento realizado sobre a família Thoracostomopsidae, pode-se observar que existe uma

discordância com as descrições de alguns gêneros. Dentre os gêneros da subfamília Enoplolaiminae, *Enoplolaimus*, *Enoploides* e *Mesacanthion* foram os que não existiam na literatura informações suficientes, seja de caráter descritivo ou ilustrativo; já os gêneros *Epacanthion* e *Oxyonchus*, são os que apresentam dados atuais e relevantes na identificação das espécies válidas. Na Bacia de Campos foram encontrados: *Mesacanthion*, *Mesacanthoides*, *Paramesacanthion* e descrição Ep*acanthion* sp. nov. Na Bacia Potiguar, a família está representada pelos gêneros: *Oxyonchus* sp, *Trileptium* sp, *Fenestrolaimus* sp, sendo os gêneros *Mesacanthion* sp e *Epacanthion* sp *es*tudados ao nível específico. Nos ambientes estudados houve uma variação na concentração dos extratos obtidos dos organismos fixados com formalina neutra e os dos organismos tamponados com bórax, porém não significativa. Entretanto, para os extratos obtidos dos organismos sem tratamento a concentração de S1 e S2 foi cerca de 78% menor. Uma variação significativa, ocorreu para os extratos obtidos dos Nematoda coletados no complexo estuarino da Bacia do Pina- PE sem formol, onde a concentração protéica foi de 80,27 µg/mL, cerca de 68% maior comparado com S3 da praia de Maracaípe. Entretanto, faz-se necessário um estudo mais detalhado, uma vez que as metodologias existentes que auxiliam a determinação de proteínas ou outras classes bioquímicas, são escassas, logo precisam ser ajustadas e adaptadas para organismos como Nematoda que apresentam um tamanho muito reduzido, dificultando assim a extração dos seus compostos orgânicos. Além disso, ainda que haja a necessidade de mais estudos a cerca da composição protéica dos nematódeos, os primeiros resultados aqui apresentados demonstraram ser esta abordagem, uma ferramenta promissora na investigação das estratégias bioenergéticas e na relevância trófica do grupo nos ecossistemas marinhos e estuarinos.

Palavras-Chave: Thoracostomopsidae, estado da arte, Bacia de Campos, Bacia Potiguar.

ABSTRACT

Free living nematodes are one of the most abundant organisms of marine and estuarine sediment. The aims of this study were: (1) to develop an update of the family Thoracostomopsidae; (2) To identify the nematode species of this family in Campos and Potiguar Basin and (3) To identify the nematofauna proteic compounds in two coastal environments. In Potiguar Basin the samples were collected in two campaigns (in 2004, with 69 stations: 43 for environmental caracterization and 26 for environmental monitoring of the submarines outfalls of Guamaré industry. In the campaigns of 2008, 36 sampling stations: 26 stattions of the submarines outfalls of Guamaré industry and 10 situated at 50 m, 200 m, 500 m, 1000 m and 2000 m from the distance of outfalls). In Campos Basin the samples were collected in nov/dec of 2002 (OCEANPROF I) and Jun/Jul of 2003 (OCEANPROF II) on board of the ship Astro Garoupa. A total of 43 stations were disposed in 9 transects depths (750m, 1050m, 1350m, 1650m and 1950m). The samples were fixed with formalin (4%) and maintained in plastic pots. In the laboratory the samples were elutriated and the nematodes were extracted. Secondly, permanent slides were made for identification generic and specific identification based on morphometry, according to the specific literature. For the proteic research, nematodes were collected from Maracaípe Beach and Pina Estuary. The samples were collected until 10cm depth with a small core and were fixed according to different treatments using pots with formalin, with sodium tetraborate 20 g and without formalin, maintained in low temperature environments. The sediment was elutriated for proteic analysis and up to 200 nematodes were picked out for each treatment. The nematodes were sonicated and centrifuged for proteic analysis using the BCA method (Protein Assay Kit). In literature, the specific descriptions of the family members studied did not present enough information for the main features. In Potiguar Basin the family is represented by the genera: *Oxyonchus sp, Trileptium sp, Fenestrolaimus sp,* where the genera *Mesacanthion sp* and *Epacanthion sp* were studied at specific level. In Campos Basin were found: *Mesacanthion, Mesacanthoides, Paramesacanthion* and described Ep*acanthion* sp nv. Different profiles were found for the proteic analysis, although further studies are needed to corroborate these results.

Palavras-Chave: Thoracostomopsidae, Updating, Campos Bay, Potiguar Bay.

SUMÁRIO

1 INTRODUÇÃO

1.1 Características gerais e taxonomia dos Nematoda

Os Nematoda são animais não-segmentados, bilaterais, a maioria com boca e ânus e, frequentemente, com uma cutícula complexa cobrindo a epiderme. Os seus representantes apresentam um corpo com 0,5-3 mm de comprimento, os menores com 0,2 mm; com comprimentos são 2-40 vezes seus diâmetros. Em termos de suas estruturas gerais do corpo, muitas características são consideradas pré-adaptadas para viver em areia e lama (GIERE, 2009). Externamente e internamente são cilíndricos, sendo, facilmente, observado: região faríngea, o meio do corpo e a cauda. Na porção ventral o poro excretor de difícil visualização, os órgãos reprodutores (vulva e espícula) e ânus ou cloaca.

No Brasil, o estudo da biodiversidade de Nematoda marinho iniciou-se em meados do século XX, sendo retomado no final desse mesmo século (CORBISIER, 1999). A partir de um levantamento, baseado na literatura disponível, existia para o Brasil até o ano de 2007, o registro de 06 ordens, 50 famílias, 291 gêneros e 231 espécies para ambientes costeiros (VENEKEY, 2007). Posteriormente, esses números foram acrescidos com a descrição de um novo gênero e uma nova espécie na Praia de Tamandaré, Pernambuco (FONSÊCA-GENEVOIS *et al*., 2009a) e uma nova espécie para uma praia arenosa da Baia de Guanabara, Rio de Janeiro (MARIA *et al.*, 2009).

Além dos ambientes costeiros, o estudo da biodiversidade de nematódeos marinhos no Brasil passou a ser desenvolvido na Margem Continental Brasileira, especialmente, na área do Talude da Bacia de Campos. Fonsêca-Genevois *et al.* (2009b) apresentou uma lista de 189 gêneros, pertencentes a 44 famílias e 9 ordens. Dentre estas, Enoplida é a mais abrangente com 13 famílias. Esse material proveniente da Bacia de Campos foi objeto de intenso estudo taxonômico, o qual resultou na descrição de 1 novo gênero e 14 novas espécies (BOTELHO *et al.*, 2007, 2009; CAVALCANTI *et al.*, 2009; GUILHERME *et al.*, 2009; LIMA *et al.*, 2009; SILVA *et al.*, 2009).

Em estudos taxonômicos de Nematoda algumas características morfológicas permitem diferenciar os indivíduos, levando-se em consideração: a cutícula, setas, o arranjo de setas

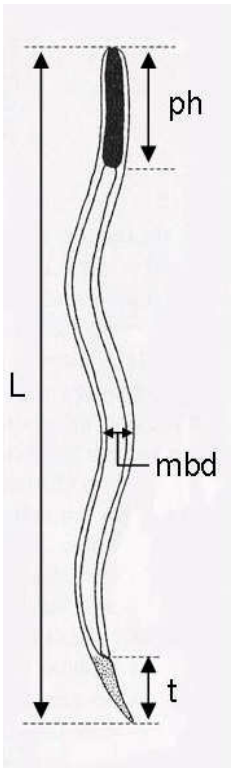
sensoriais (particularmente ao redor da cabeça), glândulas caudais, o anfídio, cavidade bucal e os aparatos reprodutores (LORENZEN, 1994; GIERE, 2009).

Além da morfologia geral do corpo, medidas corporais são essenciais para a descrição e identificação de nematódeos (COBB, 1917). Entretanto, pequenas diferenças podem ser significativas e até mesmo fatores ambientais (profundidade, tipo de sedimento, etc) podem afetar dimensões em nematódeos (GERAERT, 1968). Para cada espécime estudado são utilizadas abreviações para as regiões do corpo e as mesmas são derivadas da proposta de Coomans (1979) (Figura 1). Anterior a esta, De Man (1880) introduziu um sistema de índices que consistem de letras que designam proporções do corpo:

a: L/mbd

b: L/ph

c: L/t



Comprimento total da faringe

Comprimento total do corpo

Diâmetro máximo da região posterior do corpo

Comprimento total da cauda

Figura 1: Desenho esquemático que exemplifica as principais medidas usadas na descrição das espécies de Nematoda. Fonte: Warwick *et al* (1998), modificado por Fonsêca-Genevois *et al*. (2009b).

Para a taxonomia dos Nematoda marinhos existe uma importante ferramenta que auxilia na identificação ao nível genérico. Ainda hoje, a chave pictórica de identificação mais utilizada é aquela publicada, originalmente, por Platt & Warwick (1983). Essa chave foi, posteriormente, atualizada por Platt & Warwick (1988); Warwick *et al*., (1998). Além das chaves, existem os sítios de consulta a bibliografia, dos quais é possível destacar o NEMYS (DEPREZ, 2006). Também são encontrados bancos de dados disponíveis com informações da estrutura, dinâmica e papel funcional da meiofauna, em particular Nematoda e Copepoda (VANDEPITTE *et al.*, 2008).

De acordo com De Ley *et al.* (2006) uma das classificações mais utilizadas, até recentemente, dividia o filo Nematoda em duas classes: Secernentea e Adenophorea (LORENZEN, 1994). Essa classificação foi, originalmente, proposta por Chitwood & Chitwood (1933) e Chitwood (1937), sendo utilizada durante anos e, até mesmo recentemente, ainda fazia parte de muitos livros didáticos e na literatura especializada.

Outras propostas de classificação do Filo Nematoda, indicavam três classes para esse grupo, como por exemplo, a proposta de Inglis (1983), em função de uma possível não-monofilia para Adenophorea (DE LEY *et al.*, 2006). No entanto, nenhuma dessas propostas de classificação bi ou tripartite (duas ou três classes) estavam baseadas em princípios da sistemática filogenética (DE LEY *et al.*, 2006).

Lorenzen (1994) realizou um estudo, pela primeira vez aplicando o método cladístico, detalhado dos representantes de Adenophorea, fornecendo um sistema de classificação com base em características morfológicas. Ainda assim, apenas o uso de caracteres morfológicos seria insuficiente para solucionar todos os relacionamentos filogenéticos do Filo Nematoda (DECRAEMER & SMOL, 2006).

Kampfer *et al.* (1998) mantêm Adenophorea como monofilético, e essa suposição foi mais tarde modificada, ou outros especialistas rejeitaram a monofilia. Lorenzen (1994) preservou ‘Secernentea’ como uma classe, colocando a raiz de Nematoda entre ‘Secernentea’ e ‘Adenophorea’. Entretanto, ele enfatizou que sinapomorfias foram ausentes em ‘Adenophorea’ e vários destes taxa constituintes foram possivelmente parafiléticos.

No campo da filogenia molecular, Blaxter *et al.* (1998) produziu o primeiro sistema molecular do filo usando pequenas subunidades de sequências ribossomais nucleares (SSU). Entretanto, estas análises foram baseadas primeiramente em espécies terrestres e parasitas, economicamente importantes das Ordens Dorylaimida, Mermithida, Mononchida, Rhabditida, Trichinellida, e Triplonchida, sem incluir dados representativos de *taxa* marinhos (ex: Araeolaimida, Chromadorida, Desmodorida, Desmoscolecida, Enoplida, e Monhysterida). Além disso, estes marcadores são frequentemente, usados para distinguir entre membros do mesmo gênero ou família, não havendo informações de estudos para níveis taxonômicos mais altos (THOMAS & WILSON, 1991; POWERS *et al*., 1993; ZARLENGA *et al.*, 1998; HOBERG *et al.*, 1999; WATTS *et al*., 1999; NADLER *et al*., 2000).

De Ley & Blaxter (2002) atualizando a classificação do Filo Nematoda usando dados moleculares associados a dados morfológicos para auxiliar a colocação daqueles *taxa* em que as sequências de SSU ainda não estão disponíveis. Contudo, o sistema foi ainda baseado, em sua maioria, em *taxa* terrestres e parasitas. Com o aumento dos estudos moleculares (>200 *taxa* analisados até agora) a classificação foi confirmada (DE LEY & BLAXTER, 2004) e mantida depois da adição de numerosas espécies marinhas para a análise (MELDAL *et al*., 2007). Atualmente De Ley *et al*.(2006) classificou o filo Nematoda da seguinte forma:

FILO NEMATODA (Potts, 1932)

Classe Chromadorea Inglis, 1983
- Subclasse Chromadoria Inglis, 1983
  - Ordem Chromadorida Chitwood, 1933
  - Ordem Desmodorida De Coninck, 1965
  - Ordem Desmoscolecida Fi1ipjev, 1929
  - Ordem Monhysterida Fi1ipjev, 1929
  - Ordem Araeolaimida De Coninck & Schuurmans Stekhoven, 1933
  - Ordem Plectida Ma1akhov, 1982
  - Ordem Rhabditida Chitwood, 1933

Classe Enoplea Inglis, 1983
- Subclasse Enoplia Pearse, 1942
  - Ordem triplonchida cobb, 1920
  - Ordem Enoplida Chitwood & Chitwood, 1937
    - Subordem Enoplina Chitwood & Chitwood, 1937
      - Superfamília Enoploidea Dujardin, 1845
        - Família Enoplidea Dujardin, 1845
        - Família Anoplostomatidae Gerlach & Riemann, 1974
        - Família Phanodermatidae Filipjev, 1927
        - Família Anticomidae Filipjev, 1918
        - Família Thoracostomopsidae Filipjev, 1927

A Família Thoracostomopsidae Fiplipjev, 1927 está incluída na Ordem Enoplida por possuir uma cutícula lisa ou finamente estriada, órgãos sensoriais localizados na cutícula denominados metanemes, anfídio não espiral e um arranjo cefálico com 3 círculos de setas, sendo o primeiro e segundo círculos compostos por 6 setas e o terceiro círculo composto por 4 setas (padrão 6+6+4) (LORENZEN, 1994).

Além de apresentar estas características acima descritas, possuem uma cavidade bucal cônica, com um lábio dorsal e dois subventrais e armada com mandíbulas poderosas. Eles são formados por três placas mandibulares, um dorsal e dois subventrais que são embebidos nas paredes internas da cavidade bucal (NICHOLAS, 1993; NICHOLAS, 2007) e cada uma é suportada com um *onchium* (dente) (INGLIS, 1964). Os músculos da faríngе são presos, anteriormente, a cutícula cefálica formando uma região denominada cápsula cefálica. Lorenzen (1981) ainda cita a ausência de uma glândula cervical e a presença de duas glândulas caudais que se estendem dentro do espaço pré-anal, como característica da família (NICHOLAS, 2007).

Existe uma considerável variação morfológica da família e no grau do desenvolvimento do complexo mandibular e esta variação provem a base para separação dos gêneros nas subfamílias (WIESER, 1953). Por exemplo, a coluna mandibular pode ser forte e aquela posicionada em cada placa pode se fundir ao longo da linha mediana e suportar dois dentes fortes (como em *Enoploides* Ssaweljev, 1912) ou as colunas podem permanecer separadas com duas onchias subventrais muito grandes, projetando-se da cavidade bucal, além dos lábios, mas com a *onchia* dorsal muito reduzido (como em *Oxyonchus* Filipjev, 1927).

Os membros da família Thoracostomopsidae são animais grandes e encontrados tanto em sedimentos entremarés e infralitorais rasos (NICHOLAS, 2007). São considerados como predadores, se alimentando de turbelários e de outros pequenos animais encontrados nestes habitats citados anteriormente (GREENSLADE & NICHOLAS, 1991; NICHOLAS & HODDA, 1999). Seus representantes também fazem um papel importante regulando outras populações de Nematoda, principalmente em entremarés de praias arenosas onde eles são considerados bastante comuns (PLATT & WARWICK, 1983; GREENSLADE & NICHOLAS, 1991; NICHOLAS, 2002).

Neste trabalho, foi realizado um estudo sobre Nematoda para entender sua composição protéica, bem como, um levantamento detalhado da família Thoracostomopsidae, contribuindo assim para o incremento do conhecimento sobre seus representantes. Atualmente estudos da Família Thoracostomopsidae são raros e no Brasil existem apenas cinco registros de espécies desta família, que será descrito no capítulo 1, logo, existe uma carência de registros sobre a mesma, necessitando a realização de um estudo para conhecer um pouco mais sobre os seus representantes e entender sobre as suas variações morfológicas.

1.2 Proteínas em Nematoda

A história das proteínas começa no século XVIII, com a descoberta de que certos componentes do mundo vivo, como a clara de ovo (albúmen), o sangue e o leite, entre outras, coagulam em altas temperaturas e em meio ácido. Substâncias com esse tipo de comportamento foram denominadas albuminóides (semelhantes ao albúmen) (HANNELORE & KATHRYN, 1980).

No início do século XIX descobriu-se que os principais constituintes das células vivas eram substâncias albuminóides. Em um artigo publicado em 1838, o químico holandês Gerardus Johannes Mulder (1802-1880) usou pela primeira vez o termo proteína (do grego *proteios*, primeiro, primitivo) para se referir às substâncias albuminóides. Na verdade, foi o sueco Jöns Jacob Berzelius (1779-1848), um dos mais importantes químicos da época, quem sugeriu o termo a Mulder, por acreditar que as substâncias albuminóides eram os constituintes fundamentais de todos os seres vivos (HANNELORE & KATHRYN, 1980).

Na virada para o século XX, o interesse pelas proteínas continuava a crescer. Os químicos passaram a analisar minuciosamente essas substâncias, descobrindo que sua degradação liberava aminoácidos. Por volta de 1900 já haviam sido identificados 12 aminoácidos diferentes liberados pela degradação de proteínas. Face a essa evidência, o químico alemão Franz Hofmeister (1850-1922) sugeriu, em 1902, que as proteínas seriam formadas por aminoácidos encadeados. Em 1906 já haviam sido identificados 15 tipos de aminoácido liberados pela degradação de proteínas; em 1935 esse número subiu para 18 e, em

1940, chegou a 20, completando a lista dos aminoácidos que ocorrem naturalmente nas proteínas dos seres vivos (HANNELORE & KATHRYN, 1980).

Enquanto prosseguiam as pesquisas sobre a natureza química das proteínas, desenvolvia-se paralelamente o estudo das enzimas (Enzimologia). Em meados do século XIX já se sabia que as enzimas apresentavam semelhanças com as proteínas. Entretanto, foi somente na década de 1930 que se esclareceu definitivamente a natureza química das enzimas: todas elas são formadas por uma ou mais moléculas de proteína. Já as proteínas são as macromoléculas biológicas mais abundantes que ocorrem em todas as células. Podem ser encontradas em uma única célula, com enorme variedade de tamanho e função, e em diferentes compartimentos celulares. São polímeros de aminoácidos, e cada resíduo de aminoácido, unido através de uma ligação covalente (GELSE *et al*., 2003; LEHNINGER, 2006).

As proteínas podem ser classificadas quanto a sua composição, solubilidade, estrutura e função. A fração protéica de um organismo é composta de **proteinas hidrosoluvéis:** as que funcionam como enzimas, triosefosfato isomerase (YIMIN *et al*., 2009), receptores esteróides (HANNELORE & KATHRYN, 1980; KUMAR & LITWACK, 2009), receptores de serotonina (BLACKBURN, 2009) e moléculas carreadoras como a hemoglobina (BETTATI *et al*., 2009); de **proteínas hidrofóbicas** as quais atuam como enzimas, citocromo P450 (ISIN & GUENGERICH, 2007) além de moléculas carreadoras transmembrana tais como canais de cloro (EDWARDS & KAHL, 2010). As **proteínas insolúveis ou relativamente insolúveis** que são estruturais, colágenos (GELSE *et al*., 2003), aquelas que constituem o citoesqueleto, tubulinas (OAKLEY, 2000) e estruturas contrácteis, actina (VARTIAINEN, 2008).

As Proteínas são sintetizadas dentro de todas as células, de aminoácidos que são obtidos a partir da dieta, pela síntese ou interconversão ou por reciclagem a partir de proteínas degradadas. Vinte aminoácidos comuns são usados por células para sintetizar a maioria das proteínas; assim como muitos aminoácidos menores contribuem para proteínas especializadas, por exemplo, a hidroxiprolina no colágeno (GELSE *et al*., 2003). Uma variedade de outros aminoácidos estão presentes nas células que não são usadas na síntese de proteínas, por exemplo a β-alanina que esta presente no acido pantatênico, taurina, a qual é usada para fins osmóticos, e acido y-aminobutirico, que é usado como neurotransmissor (BEHM, 2002). Muitas proteínas são pós-translacionalmente modificadas pela glicosilação, acetilação,

miristoilação, agregação de co-fatores como FAD ou piridoxal fosfato, ou fosforilação, por exemplo (JIANREN, 2009).

As proteínas possuem quatro níveis de organização estrutural, na qual apresenta uma seqüência definida de resíduos de aminoácidos, que constitui seu primeiro nível de organização, assim chamado Estrutura primária. O próximo nível, a Estrutura Secundária, refere-se ao arranjo regular do esqueleto da cadeia polipeptídica em α-hélices, folhas β e dobras β. O arranjo tridimensional geral de todos os átomos em uma proteína é referido como Estrutura Terciária (KINCH & GRISHIN, 2002). Venyaminov & Yang (1996) relatam que algumas proteínas possuem duas ou mais cadeias polipeptídicas separadas, ou subunidades idênticas ou diferentes. O arranjo dessas subunidades em complexos tridimensionais constitui a Estrutura Quaternária.

As proteínas podem ser agrupadas em cinco classes de conformação estrutural secundária: - Toda α: Proteínas que possuem somente α-hélices como estrutura secundária; - Toda β: Proteínas formadas apenas por folhas β; - α + β: Proteínas que possuem α-hélices e folhas β freqüentemente em domínios separados; - α / β: Proteínas que apresentam segmentos intermisturados (α-hélices e folhas β) que freqüentemente se alternam ao longo da cadeia polipeptídica e – Desordenada: Incluem oligopeptídeos, polipeptídeos pequenos com pontes dissulfeto ou grupamentos prostéticos e proteínas desnaturadas (VENYAMINOV & YANG , 1996)

As proteínas de parede celular podem ser divididas em três classes estruturais. As extensinas, glicoproteínas básicas ricas em hidroxiprolina, serina, tirosina e lisina, contêm muitas seqüências repetidas de Ser $(Hyp)_4$ e são os componentes majoritários da parede celular primária, podendo conter 40% de hidroxiprolina. As proteínas ricas em glicinas são caracterizadas pela repetitividade (Gly-X), em que X freqüentemente é glicina. As proteínas ricas em prolina e hidroxiprolina contêm unidades repetidas de Pro-Pro-Val-X-Lys, em que X freqüentemente são histidina, tirosina ou glicina (KLEIS-SAN FRANCISCO &TIERNEY, 1990; WALDRON, *et al.*, 2003).

Existem alguns estudos sobre a concentração protéica em Nematoda, destacando os de Lee & Atkinson (1976) estudando Nematoda parasitas, descreveram que as proteínas constituem de 50 a 80% do peso seco destes organismos; já Danovaro *et al*. (1999) relataram

a que as mesmas representaram a principal classe bioquímica de compostos orgânicos em Nematoda (43,7 e 48,5%) costeiros e de mar profundo respectivamente, valores estes relativamente altos comparados com outros invertebrados.

Os Nematoda tendem a ser mais estudados na sua importância médica e veterinária, porque a maioria deles são pestes de culturas. Existe uma vasta literatura para nematódeos parasitas em relação a sua estrutura, sistemática, bioquímica, fisiológica, imunológica e biologia molecular (LEE, 2002). Estudos bioquímicos, envolvendo proteínas solúveis em Nematoda parasitas foram realizados nos últimos 30 anos (CARNEIRO & ALMEIDA, 2001). Entretanto, os de vida livre, já foram estudados com maior ênfase no ponto de vista ecológico, sistemático e molecular, mas estudos bioquímicos, principalmente protéicos, são raros.

Estudos sobre o metabolismo geral de Nematoda de vida livre e parasitas de plantas foram revisados detalhadamente por Barrett & Wright (1988); Chitwood (1998). Relatos gerais foram publicados por Barrett (1981; 1983; 1989) e Bryant & Behn (1989). O mais recente estudo foi apresentado por Behn (2002) realizou um levantamento detalhado sobre o metabolismo de Nematoda parasita. Porém, estudos sobre a composição bioquímica de Nematoda de vida livre são escassos. Danovaro *et al.*(1999) estudaram os Nematoda de vida livre em mar profundo e praias arenosas, levando em consideração seu valor nutricional controlado por mudanças em condições ambientais, bem como, avaliaram os efeitos do formol sobre a determinação da composição bioquímica;

Levantamentos sobre a composição bioquímica, tanto no que tange a composição e variação de dosagem de proteínas, lipídeos e carboidratos, já foram descritos para outros invertebrados marinhos. Dados prévios sobre a influência das flutuações na disposição de alimento e da temperatura sobre a composição bioquímica podem ser descritos em organismos planctônicos (BAMSTEDT, 1975, 1978; HOPKINS *et al*,. 1993) como para organismos bentônicos (ANSELL,1974; NORBBIN & BAMSTEDT, 1984; MILIOU *et al*.,1992; LEHTONEN, 1996). Barnes & Blackstock (1973) estimaram a quantidade de lipídeos em tecidos de diferentes tipos de invertebrados. Em cracas, as dosagens foram realizadas no corpo inteiro do animal (*Balanus balanoides* e *Balanus balanus*), em caranguejos no hepatopâncreas e músculos (*Carcinus maenas*) e, em mexilhões (*Mytilus edulis*) em partes moles inteiras do animal. França (2007) realizou um estudo para caracterizar

as proteases digestivas do Copepoda Harpacticoida (*Tisbe biminiensis*), bem como identificar suas enzimas digestivas.

As proteínas representaram a principal classe bioquímica de compostos orgânicos em Nematoda (DANOVARO *et al*., 1999). Através de análise em eletroforese bi-dimensional de extratos de estágios misturados de *C. elegans* revelaram pontos de gel corados em prata, representando 2000 proteínas diferentes dentro PI que variam entre 3,5-9 e o peso molecular variando de 10-200 kDa (BINI *et al*., 1997). É estimado que em media a célula eucariótica expresse 05 a10000 proteínas diferentes. A dominância de proteínas também foi observada na maioria dos estudos, quando comparada à composição bioquímica de organismos marinhos, dentre estes Copepoda (BAMSTEDT 1975, 1978); Copepoda Harpacticoida (MILIOU *et al*. 1992), crustáceos misidáceos (BAMSTEDT, 1978); Eufausiáceos (VIRTUE *et al*. 1995) e Bivalvia (ANSELL, 1974).

Estudos voltados para averiguar concentrações de proteínas em Nematoda apresentam certos interferentes. Um problema geralmente relacionado ao estudo da composição bioquímica dos organismos marinhos, principalmente em nematódeos é que para isso seria necessário que os mesmos estejam frescos ou congelados-seco. As amostras de meiofauna são comumente conservadas em formol tamponado (HIGGINS & THIEL, 1988). A possibilidade de realização de análises bioquímicas em nematódeos conservados em formol seria de grande importância, dada a completa falta de informações sobre sua composição corporal e, conseqüentemente, o papel quantitativo na transferência de energia através das teias alimentares bênticas (DANOVARO *et al*., 1999).

Para a determinação de proteínas totais, existem vários métodos, que foram desenvolvidos para diferentes amostras, tais como células bacterianas (Biureto), proteínas dissolvidas (Bradford), soluções que contenham detergentes (BCA – Ácido Bicinconínico), proteína pura (absorção UV), alimentos (Biureto, Lowry, Bradford), plasma sanguíneo (Biureto, Lowry, Bradford), plantas (Lowry), animais (Bradford) entre outras.

Cada método apresenta um princípio diferente e precisa ser analisado separadamente. O método Biureto (GORNALL *et al*, 1949) envolve a mistura de sulfato de cobre e hidróxido de sódio com tartarato de sódio, que estabiliza o cobre em solução. Segundo Zaia *et al* (1998), ocorre a formação de um complexo quadrado planar do cobre com a ligação peptídica da

proteína, em meio alcalino. Geralmente, as concentrações de proteínas são superestimadas, uma vez que todos os compostos com grupos –NH2 são medidos (ITZAHKI & GILL, 1964). O método descrito por Itzahki & Gill (1964) utiliza somente sulfato de cobre e hidróxido de sódio na formação do complexo. Apresenta limite de detecção de $1x10^{-3}$ $mg.L^{-1}$e leitura de absorbância em 310 nm (WILSON & WALKER, 1995). O complexo formado no método do Biureto também pode ser lido a 540 nm, porém com menos sensibilidade. Stickland (1951) propôs um método para determinar proteínas totais em células bacterianas com os mesmos reagentes daquele proposto por Gornall *et al* (1949), porém com posterior centrifugação para eliminar material celular e hidróxido de cobre, ambos insolúveis.

O método de Lowry apresenta limite de detecção de 0,7 $mg.L^{-1}$ e leituras de absorbância em comprimento de onda 750 nm (LOWRY *et al*, 1951). Neste método, ocorre redução dos constituintes ativos do reagente folin-fenol por meio das cadeias laterais de alguns aminoácidos que contribuem. Segundo Zaia *et al* (1998), com quatro elétrons ou pela retirada de dois elétrons de cada unidade tetrapeptídica dos peptídeos e proteínas, que é facilitada pela formação do quelato entre o cobre (II) e peptídeos/proteínas, tornando a reação biureto mais sensível. Segundo Wilson & Walker (1995), o limite de detecção deste método é de $1x10^{-5}$ $mg.L^{-1}$

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No método de Bradford (1976), as leituras são feitas a 595 nm e o limite de detecção é de $2x10^{-5}$ $mg.L^{-1}$ (WILSON & WALKER, 1995). Ocorre ligação do corante azul de Coomassie BG-250 com grupos funcionais básicos ou aromáticos das proteínas. Para isto ocorrer, a proteína deve ter estrutura macromolecular, ou seja, de 8-9 ligações peptídicas no mínimo. A ligação ocorre em dois minutos e esta dura aproximadamente duas horas (BRADFORD, 1976). Segundo Zaia *et al*.(1998), no pH de reação, a interação entre a proteína de alto peso molecular e o corante provoca o deslocamento do equilíbrio do corante para a forma aniônica, que absorve fortemente em 595 nm.

No método BCA - Ácido Bicincônico (SMITH *et al.*, 1985), com leituras a 562 nm e limite de detecção de 0,5 mg.L-1 , ocorre formação de complexo colorido com BCA, pela redução do Cu^{+2} , em meio alcalino, com proteínas (ZAIA *et al.*, 1998). A estrutura macromolecular da proteína, o número de uniões peptídicas e a presença de quatro aminoácidos particulares (cisteina, cistina, triptofano e tirosina) são registrados como os responsáveis pela formação de cores com BCA. Estudos com di, tri e tetrapeptídeos sugerem

que a extensão da formação da cor é causada por mais do que simples soma total individual-cores produzindo grupos funcionais. Concentrações de proteínas geralmente são determinadas e registradas com referência a um curva padrão de uma proteína comum com o soro de albumina bovina (BSA). Uma série de diluições de concentrações conhecidas é preparada a partir de uma proteína e são analisadas juntamente com algumas desconhecidas antes da concentração de cada uma destas seja determinada baseada numa curva padrão.

Smith *et al* (1985) declararam que este método monitora os íons cobre monovalentes produzidos na reação de proteínas com cobre bivalente, aumentando a sensibilidade do método, como ocorre no de Lowry. Existem outras substâncias capazes de reduzir o Cu^{+2} a Cu^{+1}, tais como ácido úrico e glicose que causam interferência na determinação (SMITH *et al*, 1985). Segundo Wilson & Walker (1995) o limite de detecção é de $5x10^{-7}$ $mg.L^{-1}$.

Das técnicas utilizadas para caracterização de proteínas, bem com avaliar a pureza, massa molecular de subunidades e o caráter ácido ou básico das mesmas a mais utilizada é a eletroforese. O avanço biotecnológico nos tempos modernos mostrou a relevância da eletroforese como meio de análise e purificação de produtos de natureza protéica, quer de origem animal quer de origem vegetal. Um grande número de com aplicações médicas e biotecnológicas usa a eletroforese com ferramenta metodológica essencial. Uma das contribuições que se conhece da eletroforese como meio de análise de material protéico na área médica foi a análise do plasma humano. Vários estudos para diagnosticar doenças, disfunções, inflamações, tumores malignos e anomalias cogênitas foram realizados através do pérfil eletroforético das proteínas séricas, da mobilidade eletroforétrica das hemoglobinas. Outro tipo de interesse é correlacionado a muitas enzimas industriais (lipases, celulases, xilanases, etc) que são analisadas quanto a sua complexidade protéica ou heterogeneidade por eletroforeses, nativas e desnaturantes em gel de poliacrilamida (SILVA JÚNIOR, 2001).

O uso de técnicas de eletroforese em estudos de proteínas solúveis e enzimas específicas em vários organismos tem se tornado mais populares desde os anos 1969. A teoria e aplicação do disco de eletroforese com gel de poliacrilamida foi primeiro descrito em detalhes por Ornstein (1964) e Davis (1964). A primeira pesquisa bioquímica taxonômica em nematódeos parasitas de raízes foi conduzida por Dickson *et al.* (1971) e Hussey *et al*. (1972), que empregaram discos de eletroforese para comparação de proteínas e enzimas de várias espécies de *Meloidogyne*.

Nesse método a separação das proteínas é baseada na migração destas em um gel, quando um campo elétrico é aplicado, sendo a velocidade de migração dependente da intensidade do campo, da carga líquida da proteína e do coeficiente de atrito. A eletroforese de proteínas é geralmente realizada em géis feitos de polímeros entrecruzados de poliacrilamida, que funcionam como uma peneira molecular, reduzindo a velocidade de migração das moléculas em proporção ao peso molecular de cada uma delas, por serem quimicamente inertes e devido ao tamanho de seus poros poderem ser controlados (STRYER, 1992).

O sistema desnaturantes na presença de SDS, a inbubação nas amostras protéicas o mais adequado é com SDS + agente redutor que normaliza a carga e a forma das proteínas, de modo que o elemento de distinção entre estas, quando da eletrofore, passa a ser os seus pesos moleculares. Para um grande número de proteínas, a quantidade de SDS que se prende às partes hidrofóbicas das mesmas é da ordem de 1,4g/g (SILVA JÚNIOR, 2001). O agente redutor β-mercaptoetanol ou ditiotreitol ao romper as pontes de dissulfeto presentes, em boa parte das proteínas, torna o acesso do SDS mais facilitado as partes internas das proteínas, além de contribuir para a separação de cadeia polipeptídicas mantidas por esta ligação covalente (HAMES, 2002)

2 OBJETIVOS

- Realizar um levantamento sobre “Estado da arte” da família Thoracostomopsidae com base na bibliografia existente;

- Descrever as novas espécies da família Thoracostomopsidae obtidas em amostras coletadas na Bacia Potiguar e da Bacia de Campos;

- Levantar a composição protéica da nematofauna de dois ambientes costeiros;

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Capítulos

CAPÍTULO 01

State of the art of family Thoracostomopsidae Filipjev, 1927

State of the art of family Thoracostomopsidae Filipjev, 1927

Betânia Cristina Guilherme[12], Maria Cristina da Silva[1], Alexandre Larrazábal[1] & Maria Tereza dos Santos Correa[3]

[1] Dept. Zoologia – CCB – UFPE – Cidade Universitária - Recife – Brazil - CEP 50670-420.

[2] Universidade Federal de Campina Grande – Olho d'água da Bica – Cuité - Paraíba – Brazil - CEP 58175-000. E-mail: betaguilherme@yahoo.com.br

[3] Dept. Bioquímica – CCB – UFPE – Cidade Universitária - Recife – Brazil - CEP 50670-420.

1 INTRODUCTION

The Thoracostomopsidae are large nematodes and are found both in intertidal and sublittoral sand. As predators, they are believed to feed on microturbellaria and other small animals found in these habitats (GREENSLADE & NICHOLAS, 1991). The individuals of this family possess an important role, controling other populations of nematodes, mainly in intertidal sand beaches, where they are very commons (PLATT & WARWICK, 1983; GREENSLADE & NICHOLAS, 1991; NICHOLAS, 2002).

The mainly morphological features of this family is the presence of orthometanemes, strong cephalic capsule, robust cephalic setae and buccal cavity with three mandibles and three teeth. The same authors show which the family Thoracostomopsidae is divided in three subfamilies: Thoracostomopsinae Filipjev, 1927, Enoplolaiminae De Coninck, 1965 and Trileptiinae Gerlach & Riemann, 1974 (SMOL & COOMANS, 2006).

Actually, taxonomic studies about the Family Thoracostomopsidae are rare. Initially the first studies the were accomplished Cobb (1920), Cobb (1933) and De Coninck & Stekhoven (1933).

The first contribution about the updating of marine nematodes it was by Wieser in 1953 that made a revision of the Order Enoplida Filipjev, 1927 and also revised some genera of Thoracostomopsidae, however considered this family as Enoplidae Dujardin, 1845. Following for Allgen (1959) described five new species of *Oxyonchus*.

In the decade of 70, few studies were considered important for this family: In Warwick (1970a), Warwick (1973) and Gerlach & Riemann (1974).

The major taxonomic contributions of the family Thoracostomopsidae were made in the decades of 80, 90 and 2000, that same works presented descritive data about new species, like revisions and two works about geogreaphic distributions. Some studies were published: Lorenzen (1981), Platt & Warwick (1983), Keppner (1986), Keppner (1987); Keppner (1988). In the decades of 90 ocurred the major contribution of studies from this family and published five new papers: Greesnslade & Nicholas (1991), Nicholas (1993) described two new species for Nematoda (Enoplida: Thoracostomopsidae), Lorenzen (1994) Other relevant studies for the family Thoracostomopsidae for that decade were the ones of Bussau (1995) and Nicholas & Marples (1995) The more recent study realized by Nicholas (2002), Nicholas (2004), Gagarin & Klerman (2006), Nicholas (2007) and Guilherme *et al.* (2009).

In this study will be presents a descriptive revision with a geographic distribution of the valid species of the Family Thoracostomopsidae, besides this we will make a list with the older and current key of each genera.

Studies about the family Thoracostomopsidae in Brazil can be observed in the table 1.

Table 1: Genera found in environmental studies for Brazilian coast.

Genera	Species	Author/year	Habitats	Region
Enoplolaimus	*Enoplolaimus distortus*	Gerlach, 1957a	Sandy beach	São Paulo
Mesacanthion	*Mesacanthion proximum*	Gerlach, 1957b	Sandy beach	São Paulo
	Mesacanthion rigens	Gerlach, 1957b	Sandy beach	São Paulo
Trileptium	*Trileptium stylum*	Gerlach, 1956	Ocean	Bahia
Epacanthion	*Epacanthion agubernaculus*	Guilherme *et al,* 2009	Deep sea	Campos Basin-RJ
Enoploides	-	Fonsêca-Genevois *et al.* (2009)	Deep sea	Campos Basin-RJ
Enoplolaimus	-	Fonsêca-Genevois *et al.* (2009)	Deep sea	Campos Basin-RJ
Paramesacanthion	-	Fonsêca-Genevois *et al.* (2009)	Deep sea	Campos Basin-RJ
Fenestrolaimus	-	Fonsêca-Genevois *et al.* (2009)	Deep sea	Campos Basin-RJ
Mesacanthion	-	Fonsêca-Genevois *et al.* (2009)	Deep sea	Campos Basin-RJ
Trileptium	-	Fonsêca-Genevois *et al.* (2009)	Deep sea	Campos Basin-RJ

2 MORPHOLOGY FAMILY THORACOSTOMOPSIDAE

Lips high. Only dorsolateral orthometanemes with a robust scapulas but no caudal filament (Figure 1). Inner labial sensilla robust and setiform (papilliform only in *Fenestrolaimus*), outer labial and cephalic setae robust and long epidermal glands with particularly well-differentiated outlet. Inner layer of cuticle forms a cephalic capsule on to which pharyngeal muscles are attached (Figure 2). Cephalic organs often present and of variable shape, situated frontally or ventrofrontally to the lateral setae (SMOL & COOMANS, 2006). According to Wieser (1953) variation occurs from genus to genus. In a few cases, the cephalic slit is modified and forms a cirrus (projecting club-shaped organ), such as in *Oxyonchus dentatus Ditlevsen,* 1919 (Figure 3). Amphids small and situated posterior to the cephalic capsule or absent. Amphids are typically pocket-shaped (also called stirrup-shaped or

cyathiform) fovea (Figure 4). Spacious buccal cavity with three mandibles and three teeth (one dorsal and two ventrosublateral) or with one long eversible spear (Figure 5). Female reproductive system didelphic-amphidelphic with antidromously reflexed ovaries (a single posterior ovary in *Mesacanthion monhystera* Gerlach, 1967 only). Caudal glands penetrate into the pre-caudal region (SMOL & COOMANS, 2006).

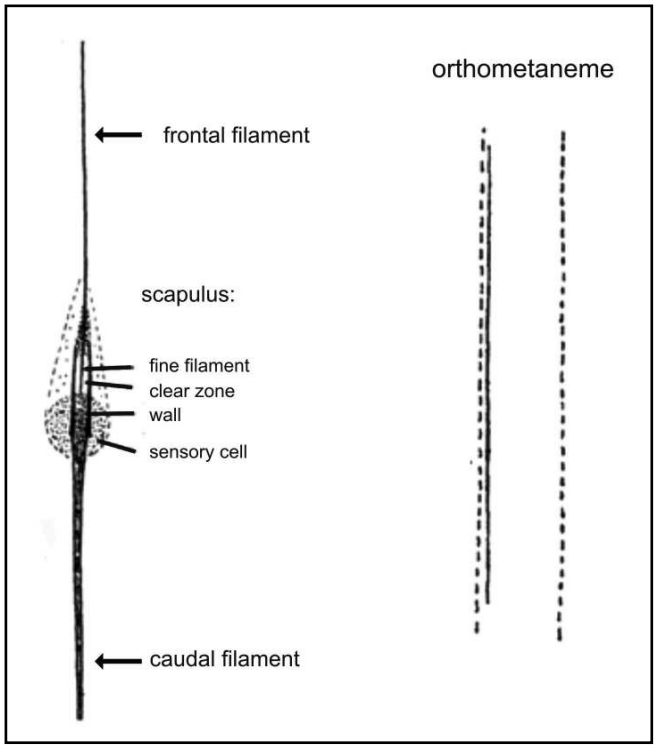


Figure 1: Dorsolateral orthometaneme with scapulas, but without caudal filament. Source: Lorenzen, 1994.

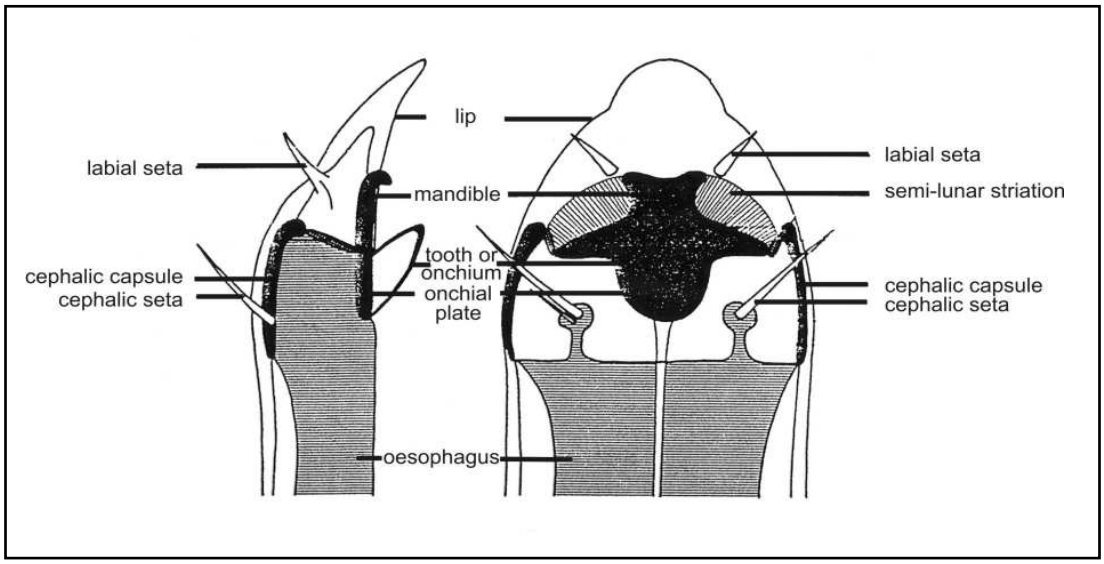


Figure 2: Structure of the head of cephalic capsule. A: longitudinal section; B: plan. Source: Smol & Coomans, 2006.

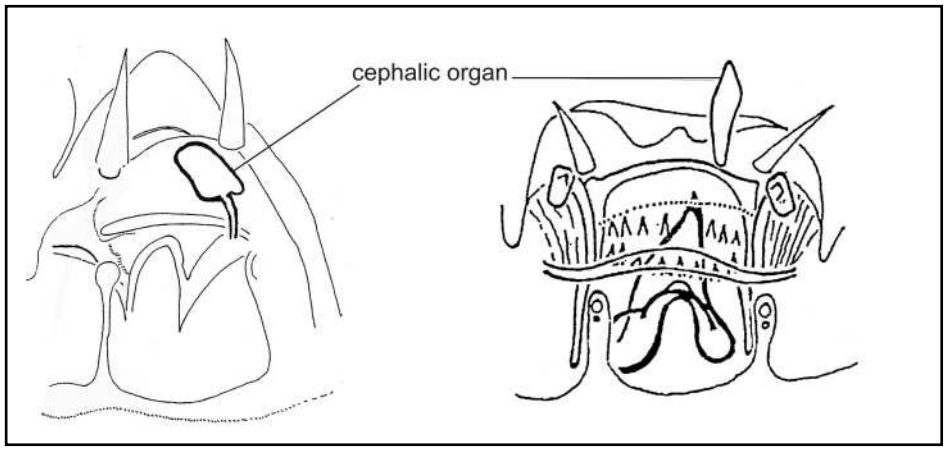


Figure 3: *Oxyonchus dentatus* showing the cephalic organ modified forming a cirrus. Source: Smol & Coomans, 2006.

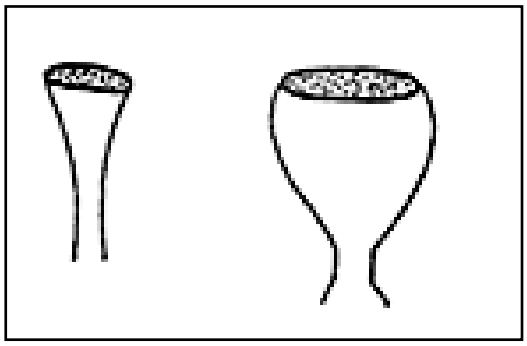


Figure 4: Two kinds of amphideal fovea pocket-shaped with a transverse slit-like or oval aperture. Source: Smol & Coomans, 2006.

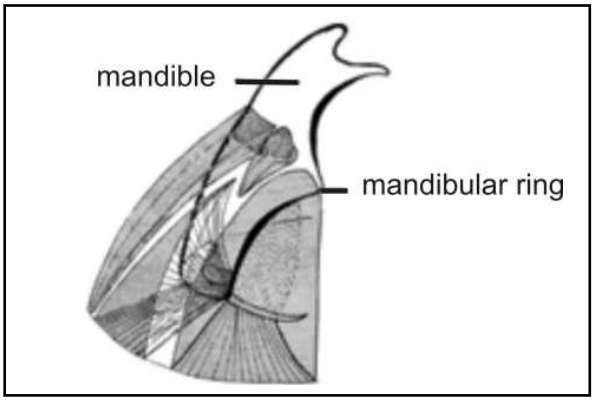


Figure 5: Mandibular plate. Source: Smol & Coomans, 2006.

3 SYSTEMATIC

Phylum NEMATODA Potts, 1932

Class ENOPLEA Inglis, 1983

Subclass ENOPLIA Pearse, 1942

Order ENOPLIDA (Filipjev, 1929)

Suborder ENOPLINA (Chitwood & Chitwood, 1937)

Family THORACOSTOMOPSIDAE Filipjev, 1927

Originally, the representative individuals from the family Thoracostomopsidae were placed in “Enoplidae” by some authors like Wieser (1953), Dujardin (1845), Platt & Warwick (1983), Platt & Warwick (1988) and Warwick *et al.* (1998). Only *Thoracostomopsis* Filipjev, 1927 was separated and the others “Enoplidae” were placed within of your own family by some authors (among then, Filipjev, 1927). Like of Enoplidae (with *Thoracostomopsis* and excluding *Chaetonema* Filipjev, 1927, probably forms a holophyletic group because: in contrast to the others Enoplida, they are unique by the presence of the mandibles and also by the presence of the onchia) onchia are absent in *Enoplus* Dujardin, 1845.

Subsequently, Thoracostomopsidae, and Chaetonematidae are removed from “Enoplidae” by the follows reasons cited by Lorenzen (1994):

a) The Thoracostomopsidae differ from *Enoplus* in the structure of the metanemes, the extension of the caudal glands well into the precaudal region, the differetiation of the outlets of the epidermal glands and the setiform condition of the labial sensilla in the first three features the Thoracostomopsidae resemble the Phanodermatidae Filipjev, 1927 extensively.

b) The Chaetonematidae differ from *Enoplus* in the form and position of the amphids and metanemes; they differ from *Enoplus* and the Thoracostomopsidae that the musculature of the pharynx has a completely different form of insertion in the buccal cavity region.

Lorenzen (1994) wrote about the holophyly of the Thoracostomopsidae that is established by the following holapomorphies:

a) Only dorsolateral orthometanemes with a robust scapulus but nocaudal filament are present (LORENZEN, 1981); this feature is unique within the Enoplina. The presence exclusivelyof dorsolateral orthometanemes occurs within the Enoplina otherwiseonly in the Oxystomininae, where, however, the scapulus is less well-developed and has a small caudal filament.

According to the same author, two features are also unique within the Enoploidea:

b) In addition to three mandibles (1 dorsal and 2 subventral), there are also three onchia in the spacious buccal cavity. This complex of features cannot be called upon to establish the holophyly of the Thoracostomopsidae, because mandibles also occur in the Enoplidae and, according to Inglis (1964: 293), onchia are absent probably at a secondary level.

c) The labial sensilla are robust and setiform in structure (papilliform only in Fenestrolaimus); since setiform labial sensilla are seen as plesiomorphic within the Adenophorea, this feature cannot be used to establish the holophyly of the Thoracostomopsidae.

Thoracostomopsidae is a family that belongs to the Order Enoplida because possess smooth cuticle, metanemes, amphids non-spiral, cephalic arrangement of 6+6+4. The subfamilies features are summarized by the presence or absence of teeth and mandibles into the buccal cavity (SMOL & COOMANS, 2006). According to Lorenzen (1994) the order Enoplida is characterised by two synapomorphies: the presence of metanemes, a smooth or only weakly striated cuticle and consists of the subfamilies Thoracostomopsinae, Trileptiinae and Enoplolaiminae. Only the subfamily Enoplolaiminae presents individuals that are representatives in freshwater environmental (SMOL & COOMANS, 2006).

Smol & Coomans revised the Order Enoplida in 2006 and criated a key for the subfamilies that is showed below:

1 Buccal cavity with a long eversible spear .....Thoracostomopsinae

2 Buccal cavity with three teeth of equal size situated well anteriorly, mandibles small or absent …….....Trileptiinae

3 Buccal cavity with three mandibles and three teeth .....Enoplolaiminae

Differences diagnosis among the subfamilies within Thoracostomopsidae (SMOL & COOMANS, 2006)

Subfamily Thoracostomopsinae Filipjev, 1927

Buccal cavity with a long eversible spear made up of the elements of the three buccal cavity sectors. Three pharyngeal glands open dorsally and ventrosublaterally at the jointed part of the spear. Marine. Type (and only) genus: *Thoracostomopsis* (Figure 6).

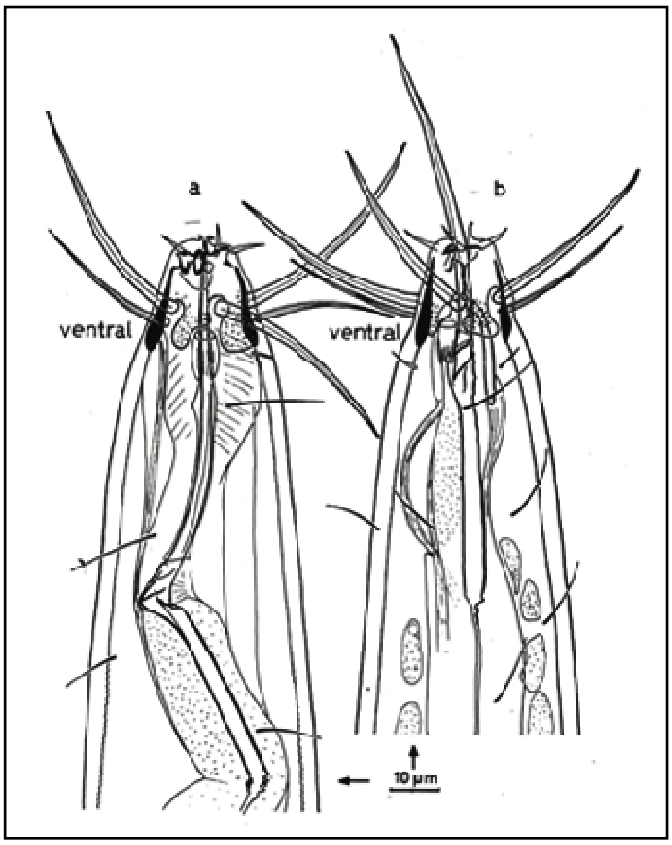


Figure 6: Anterior region with long eversible spear of species type *Thoracostomopsis barbata* Ditlevsen 1918. Source: Lorenzen, 1994.

According to Gerlach & Riemann (1974) six species were described for the subfamiliy Thoracostomopsinae: *T. barbata* Ditlevsen, 1918, *T. carolae* Inglis, 1964, *T. ditlevseni* Filipjev, 1927, *T. doveae* Warwick, 1970, *T. galeata* Filipjev, 1927 e *T. longíssima* Filipjev, 1927.

Lorenzen (1994) also wrote about the holophyly of the subfamily Thoracostomopsinae that is established by the following holapomorphy: in the oral cavity there is a long spear which is unique among the free living nematodes. According to Inglis (1964), it is made up of the elements of all three buccal cavity sectors. Inglis doubts whether the spear can justify its

name because no author has reported having seen it everted. This doubt can now be removed (Figure 6): the spear really can be everted out of the buccal cavity. Personal observations have shown that the pharyngeal glands open dorsally and subventrally on the jointed part of the spear.

Subfamily Trileptiinae Gerlach & Riemam (1974)

Three teeth of equal size are situated well anterior into the buccal cavity. Pharyngeal glands open through the teeth. Mandibles small or absent. Only two caudal glands which penetrate into the precaudal region. Marine. Type and only genus: *Trileptium* Cobb, 1933 (Figure 7).

According to Lorenzen (1994) there is known holopomorphy with which the holophyly of the Enoplolaiminae can be established. The following complex of features is very characteristic: in the oral cavity there are always three mandibles tree onchta at primary level. One mandible and one onchium together form a unit which can be moved back and forth by specialized pharyngeal muscles, whereby the frontal section of the unit is moved in line with the body axis (INGLIS, 1964). A pharyngeal gland opens through each *onchium*. In part, all three onchia are the same length, and in part, the dorsal onchium is distinctly smaller than the two subventral onchia. The two subventral onchia are always mirror-images of each other; one of the is never larger than the other, as is the case in most Oncholaimoidea.

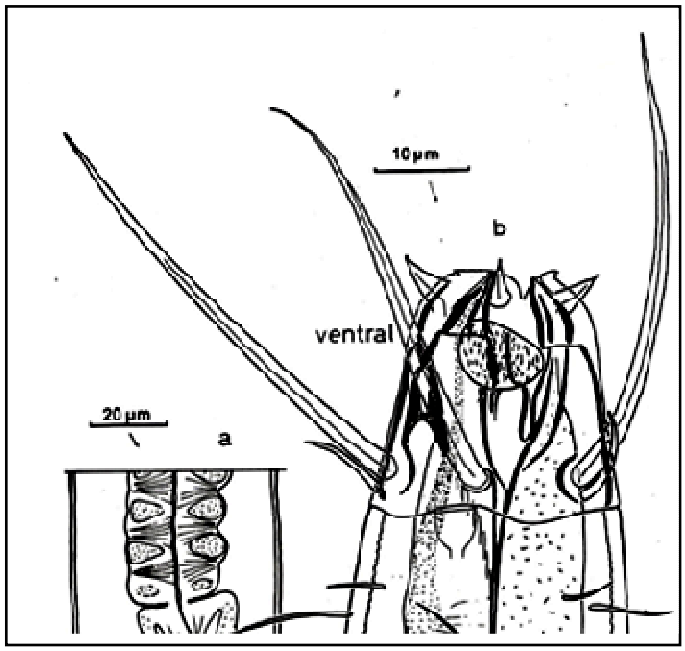


Figure 7: Anterior region and pharynx detail of type genus *Trileptium* Cobb, 1933 Source: Lorenzen, 1994.

Diagnosis of Genus *Trileptium* Cobb 1933

Body cylindrical from cephalic capsule to cloaca. Mandibles weakly developed, onchia strongly developed, the whole mandibular complex situated close to mouth opening. Lips, one dorsal and two sub-ventral, low, separated by shallow incisures. cephalic sensilla setose, in three rings: six inner labial, six outer labial and four cephalic setae (terminology from coomans (1979) based on *Caenorhabditis*). The two posterior rings are sometimes referred to as longer and shorter cephalic setae. Additional setae, inserted just posterior to the cephalic capsule, found only in males, referred to here as sub-cephalic setae to distinguish them from the shorter cervical setae found in both sexes scattered along the pharyngeal region of the body. A supplementary organ (a tubular male pre-cloacal sensilum, found in many Thoracostomopsidae, may be present or absent (NICHOLAS, 2007).

Seven species were described for Trileptiinae and are listed in Gerlach & Riemann (1974): *T. ayum* Inglis 1964, *T. guttatum* (Cobb, 1920), *T. iacobinum* Wieser 1959, *T. longisetosum* Inglis 1966, *T. salvadoriense* Gerlach 1956, *T. stylum* Gerlach 1952 and *T. subterreneum* (Gerlach, 1952).

According to Nicholas (2007) eleven species were described: *T. iacobinum* Wieser (1959), *T. guttatum* Cobb (1933), *T. subterraneum* Gerlach (1953), *T. salvadoriense* Gerlach (1955), *T. otti* (Jensen & Gerlach 1976), *T. americanum* (keppner 1987), *T. australis* Nicholas, 2007, *T. longisetosum* (Inglis, 1966), *T. parisetum* (Warwick & Platt 1973), *T. stylum* (Gerlach, 1954) and *T. ayum* (Inglis, 1964).

Taxonomic key to male *Trileptium* (NICHOLAS, 2007)

1. Cuticle annulated, gubernaculum absent .....*T. iacobinum* Wieser (1959)
   cuticle not annulated, gubernaculum present 2
2. Buccal cavityth single forwardly directed dorsal onchium 3
   buccal cavity with dorsal and two sub-ventral onchia 4
3. Supplement absent, gubernaculum without apophysis .....*T. guttatum* Cobb (1933)
   supplement present, gubernaculum with dorso-caudal apophysis.....*T. subterraneum* Gerlach (1953)
4. Supplement absent .....*T. salvadoriense* Gerlach (1955)

supplement present 5

5. Dorsal onchium does not reach mandibular transverse bar, sub-ventral onchia extend beyond bar …….....……...6

dorsal and subventral onchia at same level, extend beyond mandibular 7

6. Precloacal supplement close to cloaca (6–11 µm).....*T. otti* (Jensen & Gerlach ,1976)

precloacal supplement not close to cloaca (29 µm)..... *T. americanum* (Keppner, 1987)

7. Spicules shallow arcs, gubernaculum does not loop over specules.....8

spicules strongly curved distally, gubernacula with ventral peg, loops over both spicules.....*T. australis* Nicholas, 2007

8. Tail long and thin, 5 times body width at cloaca.....*T. longisetosum* (Inglis, 1966)

tail 4 times or less than body width at cloaca.....9

9. Gubernacula terminate in small plates each with two lateral projections..... *T. parisetum* (Warwick & Platt 1973)

spicules cylindrical tapering to point, gubernaculum reduced to knob obscuring spicule tip .....*T. stylum* (Gerlach, 1954)

Gubernaculum without terminal projections; does not obscure spicule tip..... *T. ayum* (Inglis, 1964).

Subfamily Enoplolaiminae De Coninck, 1965

Lips high. Buccal cavity always with three mandibles and three teeth. One mandible and one tooth together form a unit, which can be moved back and forth by specialized pharyngeal muscles, whereby the frontal section of the unit is moved in line with the body axis. A pharyngeal gland opens through each tooth. All three teeth have the same length or the dorsal tooth is distinctly smaller than the two ventrosublateral teeth; the two ventrosublateral teeth always are equal in length. The genera are differentiated by the degree of development of the mandible-teeth complex. This subfamily needs to be revised, as the complex structure of the buccal cavity and the head as well as the cephalic organs have been insufficiently described and misunderstood by some authors and many species need to be redescribed. Enoplolaiminae consist of 18 genera, including the genus *Hyptiolaimus*, of which the type species was considered as species inquirendum (sp. inq.) by Wieser (l953).

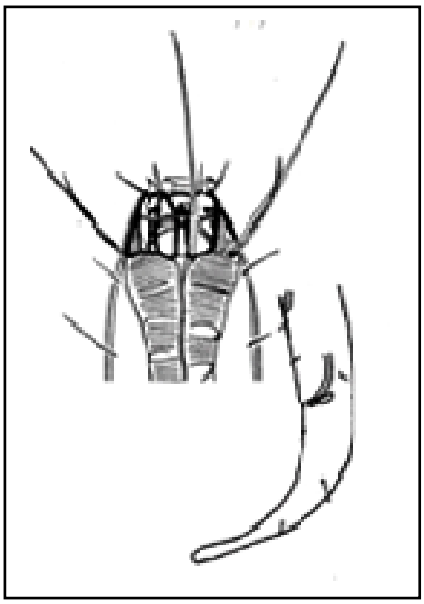


Figure 8: Type genus *Enoplolaimus* De Man, 1893. Source: Smol & Coomans, 2006.

Smol & Coomans (2006) organized a key to genera for the subfamily Enoplolaiminae. This key that is showed below is composed by 17 genera, except *Hyptiolaimus* of which the type species was considered as species inquirendum (sp inq.) by Wieser (l953).

Key to genera for the subfamily Enoplolaiminae (SMOL & COOMANS, 2006)

1. Mandibles reduced, teeth unequal,…….…..….....….....*Saveljevia*
   Mandibles existing of two longitudinal bars united by a thin sheath….....*Epacanthion*
   Mandibles solid …….....…….....2
   Mandibles arch-shaped (two longitudinal rods anteriorly united by a bar).....…….....6

2. Mandibular teeth equal.…….....…….....3
   Mandibular teeth unequal.…….....…….....5

3. Lips striated…….....…… *Enoploides*
   Lips unstriated…….....……..4

4. Mandibles extremely long, teeth reduced in size…….…..…..….. *Metenoploides*
   Mandibles and teeth of normal size,…….....….....*Mesacanthoides*

5. Dorsal tooth reduced, mandibles with long posterior apophysis…….....……..... *Filipjevia*
   Dorsal tooth absent, mandibles anteriorly with central claw in
   addition to lateral laws…...…….…….....……...*Fleuronema*

6. Teeth absent, lips plicate……..….....……*Parenoplus*
   Teeth present and equal in length …….....…..……..7
   Teeth present and unequal in length…….....……...…….11

7. Teeth arch-shaped, extending anterior to the mandibular bar……..….....*Cryptenoplus*
Teeth shorter than the mandibles…….…….....……8

8. Teeth with broad base and spine-shaped, anterior bar of mandibles forming three large anteriorly directed bows…….....…...…….*Fenestrolaimus*
Teeth not so and anterior bar of usual shape…….....……...…..…..9

9. Cuticle very thick with distinct 'shoulder'[2] outer labial and cephalic setae at posterior edge of cephalic capsule…….....….....*Okranema*
Cuticle of same thickness all over the body…….....…..…….10

10. Outer labial and cephalic setae positioned anterior to cephalic capsule, spicules in two articulating parts……...…….....……*Paramesacanthion*
Outer labial and cephalic setae positioned at middle or in anterior half of cephalic capsule…….…….....*Mesacanthion*
Outer labial and cephalic setae positioned at posterior edge of cephalic capsule…….....……..……..*Enoplolaimus*

11. Teeth slightly unequal and far posterior, males with a ventral file of stout setiform supplements……..….....…..…..*Africanthion*
Dorsal tooth small, two large ventrosublateral teeth extend anterior to mandibular bar…….....…….……12

12. Mandibular plate with denticles at inner surface…….....…….……*Oxyonchus*
Mandibular plate with or withoutdenticles…….....……..…...*Parasaveljevia*

Genus *Saveljevia* Filipjev, 1927

Mandibles reduced or vestigial Teeth unequal, two ventrosublateral ones surpassing the anterior end of mandibles, dorsal tooth small or reduced. The cephalic ring, however, can be very well developed. Marine (SMOL & COOMANS, 2006). Besides the mandible to be vestigial or reduced, the cephalic ring, however, can be very well developed (WIESER, 1953). Four species are known according to Wieser (1953) and is showed in the table 2. The key below belongs to the same author.

A. Cephalic setae implanted in the middle of the cephalic capsule…….…….....……..*S. xiphonchus*

B. Cephalic setae implanted at the anterior end of the cephalic capsule.

1. Cephalic setae more than half of the head diameter long, teeth straight.…….…..…….....….*S.hastata* Wieser (1953)

2. Cephalic setae not longer than half of the head diameter; teeth curved.
a. Cephalic setae one fourth of head diameter long; cephalic ring interrupted *S. kolaensis*

b. Cephalic setae one half of head diameter long; cephalic ring uninterrupted…….....……*S. curvidens*

This species above is most probably synonymous with *Enoplolaimus spissignathus* Allgen 1940. The same author misunderstood the structure of the buccal cavity completely and confused the teeth with the mandibles.

Genus *Epacanthion* (Wieser, 1953)

Cuticle usually smooth. Head broadly wedge–or cone–shaped. Lips high, mostly striated. Inner labial setae long and inserted at the base of lip flaps; outer labial and cephalic setae situated at middle or anterior end of cephalic capsule. Cervical setae often present and can be numerous in males, which exhibit sexual dimorphism. Mandibles consisting of two plate–shaped columns (usually long and parallel) separated by a thin sheet of cuticle (space between columns not solid) and only connected anteriorly by a bar (an intermediate stage between *Enoploides* and *Mesacanthion*); mandibular teeth small with gland opening at tip. Pharynx relatively long and cylindrical; cardia pyriform. Females didelphic–amphidelphic with reflexed ovaries at left side of the intestine. Males diorchic with both testes at left side of the intestine; Spicules mostly long (≥ 2.5 anal diameters long) or short; gubernaculum without apophyses present or absent. Pre-anal supplement present or absent. Three caudal glands, cells pre-caudally. Tail narrowly conical or attenuated. Marine. (SMOL & COOMANS, 2006).

Key to species of *Epacanthion* (GUILHERME *et al*., 2009) (Table 3)

1 Spicules without gubernaculum.....2
- Spicules with gubernaculums.....3

2 Spicules long (more 100 μm), one pre-anal supplement..... *E. agubernaculus*
- Spicules short (less 100μm), without pre-anal supplement..... *E. oliffi*

3 Spicules short, 2-5 or fewer anal diameters long.....4
- Spicules long, 3 or more anal diameters long.....14
- 4 Pre-anal supplement present….....5
- Pre-anal supplement absent.....12

5 Nine to 14 small, setose pre-anal supplements.....6
- Only one pre-anal supplement.....7

6 Distance between tips of apical teeth about equal to length of mandibular columns, which are short, stout and parallel..... *E. multipapilatum*
- Mandible with tips of apical teeth much closer together than length of mandibular columns, which are slender and diverge at apex..... *E. oweni*

7 Distance between tip of apical mandibular hooks either clearly much greater or much less than length of mandibular columns.....8
- Distance between apical mandibular hooks apparently about same as length of mandibular columns.....*E. brevispiculum*

8 Distance between tip apical mandibular hooks much greater than length of mandibular columns.....9
- Distance between apical mandibular hooks much less than length of mandibular columns.....10

9 Spicules 1–2 anal diameters (59 μm), gubernaculum a triangular plate, dorsal onchia much smaller than other two, 12 pairs of cervical setae in male, 4 in female..... *E. mawsoni*
- Spicules 0–75 anal diameters (34 μm), gubernaculum reduced, onchia apparently unequal, male with 12 cervical setae (female unknown)..... *E. pellucidum*

10 Spicules 2–5 anal diameters (80 μm), gubernaculum present (37 μm) lip not striated..... *E. microdentatum*
- Spicules 1 anal diameter (90 μm), lip flaps striated.....11

11 Small species (1.5–2.0 mm long), male head hirsute, cephalic setae maximum length 30 μm, inserted above cephalic arch, posterior rim of cephalic capsule crenelated (indistinct)... *E. galeatum*
- Larger species (2.2–2.9 mm long), male head hirsute, cephalic setae maximum length 50 μm, longer than head diameter, inserted just below cephalic arch, posterior rim of cephalic capsule not crenelated..... *E. exploratoris*

12 Male head hirsute with many subcephalic and cervical setae..... *E. enoploidiforme*
- Male head not hirsute.....13

13 Mandibles very long and slender, mandibular columns about twice as long as distance between apical teeth..... ……..……..…*E. gorgonocephalum*
- Mandibles not long and slender, distance between mandibular teeth less, or only slightly longer (1.2x), than mandibular columns..... *E. georgei*

14 Tail long, 4 or more anal diameters, spicules of equal length, may be annulated.....15
- Tail short, only 3 anal diameters, spicules always annulated, may be of unequal length.....18

15 Spicules >200 μm (3–3. 5 anal diameters), always annulated.....16
- Spicules 130–175 μm (3–3. 5 anal diameters) long, not annulated.....19

16 Larger species, males and females more than 4 mm long, α value 27–34, onchia nearly reaching cephalic arch, inner cephalic setae long, 40 μm.....17
- Smaller species, 2.9–3.1 mm long, α value 117–19, onchia small, not reaching cephalic arch, inner cephalic setae very short, 28 μm..... *E. durapelle*

17 Tail setae numerous (>10 setae)..... *E. buetschlii*
- Tail setae sparse(only two medial setae)..... *E. stekhoveni*

18 Spicules of unequal.....*E. nadjae*
- Spicules equal in length..... *E. saveljevi*

19 Head blunt anteriorly, mandibular columns parallel, diverging strongly at apex, male supplement 90 μm in front of anus.....*E. brevispiculosum*
- Head pointed anteriorly, mandibular columns parallel, male supplement 132–158 μm in front of anus.....*E. polysetosum*

Genus *Enoploides* Ssaweljev, 1912

Lips high, striated. Buccal cavity with well-developed solid mandibles with claw-like anterior; mandibles not extremely slender (ratio length/width <6); teeth shorter than the mandibles; spicules usually long, Mostly marine, two freshwater species (SMOL & COOMANS, 2006). Type species: *E. typicus* Ssaweljev, 1912. According to Gerlach & Riemann (1974) exists 34 species described for this genus, but actually already were 07 new species described and among then 03 were not gotten any information (Table 4) Nevertheless, are lacking more significant data a main grouping of most of the species according to the length of the cephalic setae is given below:

1. Filipjev (1925, p. 141) states that several species of the genus under consideration are devoid of teeth.

All species hitherto known are actually in possession of teeth fixed basally to the mandibles, though they might sometimes be very small.

2. Brunetti (1950) stresses the discrepancy in several descriptions as to the number of cephalic setae. However, this discrepancy is attributable to the fact that the setae of the submedian pairs are liable to stick together and so easily can be taken for one single seta (see also above, *Oxyonchus dentatus*). At least this explanation seems to hold good in cases where species are described either with 6 or with 10 setae.

Wieser (1953) agrees with Brunetti op cit about the questionable identity of the numerous specimens described under the name of *E. labiatus* is concerned, though exists doubts that the authors are not based on the different number of cephalic setae in the different descriptions (BUETSCHLI, 1874; SCHULZ, 1932; STEKHOVEN, 1935; BRESSLAU & STEKHOVEN, 1940) but on the relative length of the labial setae, length of tail etc. However, the present data are too scarce to clear up the whole question. The all species are showed in table 4. Below, follows the reproduction of the key that was proposed by Wieser (1953).

A. Anterior end of mandibles with three tips:
E. tridentatus Saveljev 1912

B. Anterior end of mandibles with two tips:

1. Cephalic setae measuring one third of head diameter:
E. brevis Filipjev 1918

2. Cephalic setae measuring 2 head diameters:
E. tyrrhenicus Brunetti 1949

3. Cephalic setae measuring between 0.5 and 1 head diameter.

a. Cephalic setae measuring between 50% and 55% of head diameter:

aa. Spicula much longer than tail:
E. labrostriatus (Southern,1914)=*Enoplus l.= E. arnphioxi* Filipjev, 1918: The author comments: “There is no difference between the two species; when Filipjev wrote the systematic part of his paper, the contribution of Southern was apparently unknown to him since he does not list Southerns’s two species in his key.
Length=5-7 mm; mandibles with basal apophyses.
E. hirsutus Filipjev1918: Length=2,8 mm; mandibles without basal apophyses.

bb. Spicula much shorter than tail:
E. rnurrnanicus Saveljev, 1912
b. Cephalic setae measuring two thirds of head diameter:
E. brattströrni Wieser, 1953

c. Cephalic setae measuring three fourths to one head diameter.

aa. Head spherical; cephalic setae equal in length*: E. cephalophorus* (Ditlevsen, 1919)= *Enoplolairnus cephalophorus.*

bb. Head not spherical; cephalic setae unequal in length.

aaa. No supplement, 7-8 preanal papillae: *E. cirrhatus* Filipjev 1918

bbb. Supplement present, no preanal papillae

(Of the species belonging to this group some are insufficiently described and some known as females only; it is difficult to ascertain their respective status unless new data are procured):

E. labiatus (Buetschli 1974): *Enoplus labiatus*: *E.spiculohamatus* Schulz 1932.
E. typicus Saveljev 1912

E. pellucidus Saveljev 1912: the specifications in the table below are based on a male and a female in my collection which do not contradict the few remarks given by Saveljev
E. paralabiatus Wieser, 1953, *E. reductus* Wieser, 1953, *E. longicaudatus* Wieser, 1953

The following species are doubtful:

E. italicus (Steiner, 1921) = *Enoplolaimus italicus*: only figure of the head without description; 12 setae!
E. longisetosus Stekhoven, 1943: only one juvenile known; very long cervical and body setae.
E. balticus Stekhoven, 1935 = *Enoplolaimus savelfevi* Allgen 1929e: almost certainly juvenile of *E. labiatu*s.
E. macrochaetus (Allgen,1920) = *Enoplolaimus macrochaetus.*
E. sabulicolus (Allgen, 1933b) = *Enoplolaimus sabulicolus.*

Genus *Metenoploides* Wieser, 1953

As the name already suggests this genus is a further development of *Enoploides*. Main characteristic is the extreme length of the mandibles which exceeds at least 10 times the corresponding width at the middle of the shaft; furthermore, the lips are very high and deeply cut and the longest cephalic setae measure 2 head diameters or more. The teeth are very much reduced in size. Unfortunately, males are not known. Exist only two species described for this genus (Table 5).

M.alatus Wieser, 1953. Head diameter = 58% of diameter at end of esophagus; submedian pairs of cephalic setae 55+23 µ long. Type.

M. capitulum Wieser, 1953 Body tapering exceedingly towards the head, the head diameter being only 16% of the diameter at the end of the esophagus; cephalic setae 17+6,5 µ long.

Genus *Mesacanthoides* Wieser, 1953

Transition between *Mesacanthion* and *Enoploides*. Mandibles solid with claws. Teeth shorter than mandibles. Marine (SMOL & COOMANS, 2006). The lips are not striated and the general appearance of the head is as in *Mesacanthion* while the mandibles are quite differently built, i.e. they are not arch-shaped but solid as in *Enoploides*. As to the male genital apparatus there is a great difference betweenn the two species reckoned to this genus,

i.e. *M. latignathus* and *M. sculptilis*, since in the former it is very much reduced, in the latter, however, extremely well developed (WIESER, 1953). According to Wieser, there are two species described (Table 6). Type species *M. sculptilis* Wieser, 1953

M. latingnathus (Ditlevsen 1919) = *Enoplolaimus latingnathus*. Accessory piece very small, supplement reduced; submedian pairs of cephalic setae of equal length.

M. sculptilis Wieser, 1953 - Accessory piece big, with dorsal apophysis, supplement strongly developed; submedian pairs of cephalic setae unequal in length.

Genus *Filipjevia* Kreis, 1928

Lips high, all anterior sensilla setose. Mandibles solid with posterior apophyses and with teeth. Dorsal tooth reduced. Two large ventrosublateral teeth not surpassing the mandibles. Marine (SMOL & COOMANS, 2006). Type and only one species: *F. macrolabiata* Kreis, 1928 (Table 7).

Genus *Fleuronema* Greenslade & Nicholas, 1991

Large specimens, head blunt but apically rounded, bulbous, with distinct external groove at base of head capsule; lip flaps low and finely striated; lips low; mandibles solid, with central tooth in addition to two lateral apical teeth, each with an apical lateral projection; sub-ventral onchia large, blunt and slanting, reaching as far as cephalic arch, dorsal onchium absent; onchial glands not particularly well developed; labial setae short and stout; cephalic setae long and slightly clavate, with surface ornamented by meshwork, inserted just above base of head capsule; posterior margin of head capsule indistinct; oesophagus long and cylindrical; cardia pyriform; nerve ring about length of oesophagus from buccal cavity; cuticle thick and smooth; female gonad on left of intestine, outstretched; spicules heavy and encircled by a distinct ridge which divides spicules in half; gubernaculum and pre-anal supplement present; three pre-anal caudal glands; tail conical, with rounded tip.

This genus is distinguished from all other genera in the Throacostomopsidae by being the only one known so far with a central tooth on the mandible. Two species are known, both from Australia. A related genus, *Paramesacanthion* Wieser 1953, has a divided spicule butno

central tooth to the mandible. Greenslade & Nicholas (1991) revised some genera of the Family Thoracostomopsidae and listed three species (Table 8). Type species *F. dorca* Greenslade & Nicholas, 1991. The same authors made some remarks about this species that is showed below:

F. dorca Greenslade & Nicholas, 1991 - is clearly distinguished from *F. robusta* by its more slender mandibles, as given in the key. Supplement present; three pre-anal caudal glands; tail conical, with rounded tip.

F. robusta Greenslade & Nicholas, 1991 - mandible square with granular surface; cervical setae consisting of a whorl of 16 pairs of very long fine setae and very short fine setae; body setae fairly abundant, with whorls of 8 pairs of very fine setae, about 23 whorls between cervical setae and oesophageal valve; spicules with thick wall, but long and thin, with hooked tip, 1/6 anal

F. tridentatus Greenslade & Nicholas, 1991 = *Enoploides tridentatus* Ssaweljev, 1912:116 - The mandible was described as having a third apical tooth centrally and, on the basis of this, the species is placed in the new genus *Fleuronema*.

Genus *Parenoplus* Filipjev, 1927 (Table 9)

Lips high and plicate, Inner and outer labial and cephalic sensilla setiform, Cephalic capsule short, Mandibles arch-shaped without claws and teeth (visible in juveniles only), Marine (SMOL & COOMANS, 2006). Only two species known. Wieser, 1953, comments that seems to be a discrepancy between the type species and *P. serratus* described by Wieser: in *P. edentates* the mandibles are arch-shaped and in *P. serratus* the mandibles are solid. Furthermore, there is a lapsus in the size of the cephalic setae of *P. edentatus* as mentioned by Wieser (1953): according to Filipjev (1927) the six outer labial setae are 25 µm and the four cephalic setae are a little shorter (so not 8 µm as mentioned by Wieser, 1953). Type species *P. edentates* Filipjev, 1927.

The differences between the type-species and that *P. serratus* is showed in the table 9. Wieser (1953) believes that *Enoplolaimus philippinensis* Allgen 1951 belongs to this genus.

However, the figure and description of this species are confusing and insufficient, so that it is impossible to make any further suggestions.

Genus *Cryptenoplus* Riemann, 1966

Three high striated lips, Mandibles existing of two strongly sclerotized lateral rods with claws anteriorly and a weakly sclerotized part situated interlabiaL Teeth modified into narrow arch-shaped tooth-like structures, which rise above the mandibles, Sub-cephalic setae present. Marine (SMOL & COOMANS, 2006). Type and only one species: *C. gerlachi* Riemann, 1966 (Table 10).

Genus *Fenestrolaimus* Filipjev, 1927

Body strongly attenuated anteriorly. Cuticle smooth. Tail conicaL Amphid pocket-shaped with round aperture. Mandibles arch-shaped, the edges of the arch thinly sclerotized. Three teeth broad at the base of the buccal cavity and strongly attenuating anteriorly to become spine-shaped. Marine. (SMOL & COOMANS, 2006).

However, Lorenzen (1994) commented which the strange fact that the descriptions were made only with females and juveniles. There are 3 species described. Type species *Fenestrolaimus insulaeabae* Filipjev, 1927 (Table 11)

Genus *Okranema* Greenslade & Nicholas, 1991

Short, broad, slightly hook-shaped when preserved, with square head and very thick, annulated cuticle, with distinct shoulder at base of head capsule, where the cuticle sharply increases in thicknes; lip flaps well-developed and with fine ridges forming striations along their edges; cephalic setae inserted just above base of head capsule; short sub cephalic setae, mandibular teeth S- or hook-shaped, mandibular columns distinct, arch anteriorly between teeth; labial setae fairly long but stout; oesophagus cylindrical; cardia pyriform; female gonad lying to left of intestine; spicules with thick walls, about as long as anal diameter; S-shaped gubernaculum, pre-anal supplement absent; caudal glands pre-anal; extremely short broad tail.

The mandibles of the two species in this genus are not as solid medially as those of *Enoploides*, but more so than in *Epacanthion*. Species of *Okranema* differ from those of *Epacanthion* in the shape of the head, the presence of 'shoulders', the thick cuticle and the S-shaped gubernaculum. The two species described were at first thought to form a separate species-group within *Epacanthion*, but the erection of a new genus for them is justified by the array of characters that distinguishes them from *Epacanthion*.

The genus is distingished from other genera of Thoracostomopsidae by the combination of head shape, cuticle, form of the mandibles and gubernaculum, and by the short, broad, tail, as given in the key. The thick, hyaline cuticle behind the head swells even more on fixation (the condition shown in the figures). It was found two species described in literature (Table 12). Type species: *Okranema eileenae* Greenslade & Nicholas, 1991

Genus *Paramesacanthion* Wieser, 1953

Outer labial and cephalic setae in front of anterior end of cephalic capsule, about the level of anterior end of mandibles". Sub-cephalic setae at middle of cephalic capsule". Mandibles arch-shaped and with claws, consisting of two pieces united by an anterior bar only. Teeth shorter than mandibles. Spicules consisting of two portions, a distal and a proximal one articulating with each other. Males with or without supplement. Sexual dimorphism in the pilosity of the head, Marine (SMOL & COOMANS, 2006). According to Gerlach & Riemann (1974) exists 15 species described. Type species: *P klugei* (Filipjev, 1927) Wieser, 1953 (Table 13).

Key to species of *Paramesacanthion* (Warwick, 1970b)

1. *a* Spicules shorter then 1-2 cloacal diameters……...…….....…….…..…..…..…..…..*2*

   b Spicules longer then 2-3 cloacal diameters…….....…..…….4

2. *a* Supplement present in male…….....……*P. allgeni* Mawson, 1958

   b Supplement absent..…….....…….....3

3. *a* Dense groups of cervical setae behind cephalic capsule in male, no constriction between spicule joints……..…….....……*P. hirsutus* Warwick, 1970b

b No dense groups of cervical setae, sharp constriction between spicule joints……..…..…*P. oxycephalus* (Ditlevsen, 1926) = *Enoplolaimus oxycephalus.*

4. *a* Gubernaculum a small plate at the distal end of the spicules…….....…..…..…...…..…5

b Gubernaculum long and tubular, about half the spicule length…….....……6

5. *a* Spicules 2.5 cloacal diameters long, distal portion narrower than proximal……...…...*P. tricuspis* (Schuurmans Stekhoven, 1950) = *Mesacanthion tricuspis.*

b Spicules 3-4 cloacal diameters long, proximal portion narrower than distal…….....…….....…*P. klugei* Filipjev, 1925

6. *a* Spicules with prominent recurved barbs subterminally, tail with two whorls of long terminal setae……..……..... *P. barbae* Inglis, 1967

b Spicules not barbed, tail with only four terminal setae none at all …….7

7. *a* Supplement level with or anterior to spicules tips…..…..... *P. marei* Warwick, 1970b

b Supplement between joint and proximal tip of spicules…….....8

8. *a* Spicules 2.4 cloacal diameters long, no cervical setae in groups …….....……...*P. estridia* Wieser, 1953

b Spicules 4-5 cloacal diameters long, oesophageal region with dense groups of cervical setae…….....…*P.inaequalis* Wieser, 1953

Key to species of *Paramesacanthion* (Boucher, 1970)

1. Absence of the cervical setae group.

Longer cephalic setae: 10 µm.
Spicules of 90 µm = 2.4 anal body diameter..... *P. estridia* Wieser, 1953

2. Presence of the cervical setae group.

Cephalic setae longer than 15 to 17 µm.

a) Spicules of 180 µm = 4.5 anal body diameter. Four groups of 4 cervical setae of 18µm, located 5 times of head diameter, in the male anterior region. Female with 6 setae of 20

µm situated abou tone head diameter.....*P. inaequalis* Wieser, 1953.

b) Spicules of 50-60 µm.

c) Spicules of 50 µm = anal diameter. Preanal tubular gland situated at 110-120 µm from the anus. Some cervical setae.....*P. allgeni* Mawson, 1958

d) Spicules of 60 µm = 1.5 anal diameter. Preanal tubular gland situated at 50-60 µm from the anus. Males with 4 groups of 12 cervical setae with 12 µm long situated two times of head diameter, start in anterior region. Absence of cervical setae in females.....*Paramesacanthion catellus* Boucher, 1970

Genus *Mesacanthion* Filipjev, 1927

Outer labial and cephalic setae situated at middle or anterior end of cephalic capsule. Mandibles well developed, provided with claws, arch-shaped, consisting of two rod-like columns anteriorly united by a curved bar. Teeth shorter than mandibles. Spicules mostly short, if long (*M. diplechma*) then gubernaculum with caudal apophysis. Marine (SMOL & COOMANS, 2006). 37 species belonging to this genus are separated from the other ones by several characters (Table 14). Type species *M. luciferum* (Filipjev, 1927).

Wieser (1953) separated the females in the genus *Mesacanthion* in two groups: with asymmetrical ovaries, that the only one species is *M. lucifer* Filipjev, 1925 and the others species with symmetrical ovaries. Besides this, it was divided the males with spicules with 8 anal diameters long that the only one species is *M. diplechma* (Southern, 1914). The others spicules is not longer than 2 anal diameter. The author also distinguished two groups according to the shape of the buccal cavity that is together with others features followed:

A. Buccal cavity almost cylindrical in the posterior part, with rounded pockets at the base.
1. Tail blunt, conical:
a. Cephalic setae one third of head diameter long:
M. hawaiiensis (Allgen, 1951) = *Enoplolaimus hawaiiensis.* Insufficiently described.

b. Cephalic setae four fifth of head diameter long or more:
M. infantilis (Ditlevsen, 1930) = *Enoplolaimus infantilis* = *Enoplolaimus mortens*eni Allgen 1951.

No ocelles, no supplement.
M. virilis (Ditlevsen, 1930). *Enoplolaimus virilis* With ocelles, with supplement.

2. Tail slender, posterior third almost filiform:
M. pacificus (Allgen, 1947), redescription 1951 = *Enoplolaimus pacificus.*

B. Buccal cavity conical, no pockets.
In this group 3 species are included which are known as females only. They can be distinguished from the other species by the following characters:

M. conicus (Filipjev, 1918) = *Enoplolaimus conicus*: Cephalic setae only 0.3 head diameter long.
M. banalis Filipjev 1925: Labial setae very short (6 µm) cephalic organ very large and oblique.
M. ungulates Wieser, 1953: Labial setae very long and slender (in juveniles 15 µm = 0.5 head diameter); lips very high (17 µ); mandibles with strong claws.

In the remaining species the labial setae are at least 10µ, the cephalic setae at least 0,5 head diameter, long; on the other hand the combination of characters as in *M. ungulatus* does not occur in them. Since they represent a fairly uniform group no key will be given of the species in question and the reader should consult the original descriptions. The following species are reckoned to this section:

M. ditlevseni Filipjev, 1925 = *Enoplolaimus angustignathus* Ditlevsen, 1928.
M. breviseta Filipjev, 1925. *M. maior* Filipjev, 1925b. *M. karensis* Filipjev, 1925
M. audax Ditlevsen, 1919
M. longissimesetosus Wieser, 1953 is distinguished from the above-mentioned species by the long cephalic setae, being in adults 1.6 head diameters long, by the weakly developed dorsal apophysis of the accessory piece and by several other minor characters, Doubtful species are the following:
M. donsi-tarvae (Allgén, 1935) = *Enoplolaimus d donsi-tarvae* The author mentions proximity to *M. audax*, but gives no figures. I think the species should be regarded as identical with the latter since the main distinguishing character, i.e. the number of cephalic

setae, does not hold good, as it is based on the erroneous data of Ditlevsen for his *M. audax.* Both species (as all *Enoplidae*) possess 10 cephalic setae.

M. gracilisetosus (Allgen, 1930) = *Enoplolaimus gracilisetosus*
M. pamdentatus (Allgen, 1932) = *Enoplolaimus Pamdentatus*
M. primitivus (Allgen, 1929) = *Enoplolaimus primitivus*

Genus *Enoplolaimus* de Man, 1893

Cuticle smooth or striated and punctated, Buccal cavity with mandibles with claw-like anterior; mandibles arch-shaped, consisting of two pieces, which are united by an anterior bar only. Teeth shorter than the mandibles. Outer labial and cephalic setae situated at posterior end of cephalic capsule, Marine (SMOL & COOMANS, 2006).

Being almost 30 years ago since Filipjev (1925) split up the original genus of De Man's into three new well-defined subgenera (which later on were given generic rank by De Coninck & Stekhoven, 1933): *Enoplolaimus*, *Mesacanthion* and *Oxyonchus.* Allgén, however, apparently does not acknowledge the validity of Filipjev's distinction and in his numerous papers till the last one called *Enoplolaimus*, what is actually a confusion of this genus with *Mesacanthion*, *Oxyonchus*, and sometimes even *Saveljevia*, *Parenoplus*. There are 84 species described (Table 15). Type species *E vulgaris* De Man, 1893.

The genus is very homogenous and in some eases the identity of, or distinction between, different species can not be cleared up and has to be postponed until further material is procured. 32 made the key that followed below:

According to Wieser (1953):

A. Head with four circles of cephalic setae and numerous sub cephalic setae:
E. caput-medusae Ditlevsen, 1919

B. Only two circles of setae (=labial and cephalie setae) and a few or no subcephalie setae present:

1. Nerve-ring at two thirds of length of esophagus:

E. abnormis Kreis, 1928

2. Nerve-ring in front of middle of esophagus:

a. Body setae extremely long (up to 100 µm):

E. psammae Gerlach, 1952

b. Body setae mueh shorter.

aa. Tail 10 anal diameters long or more:

E. longicaudatus (Southern, 1914) = *Enoplus longicaudatus.*

E. conicollis Gerlach, 1952

bb.Tail less than 7 anal diameters long.

aaa. The longest cephalic setae (of female) not longer than I head diameter:

E. vulgaris De Man, 1893

bbb. The longest cephalic setae measure at least 1.5 head diameters:

&. The four shorter submedian setae 1.5 head diameters long:

E. halophilus Ditlevsen, 1928

& &. The four shorter submedian setae not longer than I head diameter:

§. The lateral setae measure 1.6-1.9 times the length of the longest submedian setae:

E. litoralis Schulz, 1936.

§§. The lateral setae not much longer than the longest submedian ones:

+ Sub cephalic setae in the male longer than the head diameter; spicula straight, fused in the distal half; accessory piece reduced (or fused with the spicula?):

E. connexus Wieser, 1953

+ +, Subcephalic setae not longer than the head diameter; spicula curved, not fused; accessory piece distinct: To this group belong the following 5 species which are very closely related and sometimes only vaguely distinguishable from each other.

The following species are of doubtful position (by Wieser, 1953):

E. similis Allgen 1929 and *E. parapropinquus* Allgen 1949 are most probably synonymous with *E. vulgaris*.

E. conicaudatus Allgen, 1929. *E. strandi* Allgen, 1940.

E. disasteri Allgen, 1951, most probably does not belong to *Enoplolaimus*.
Enoplus filiformis Allgen, 1935, on the other hand, may belong to *Enoplolaimus*.

Genus *Africanthion* Inglis, 1964

Mandibles with lateral processes (claws) very well developed and mandibular walls fairly narrow in optical section; mandibular plate thin; teeth slighrly unequal: dorsal smaller than ventrosublateral; teeth lying far posterior to mandibles, Cephalic setae arising from the middle of cephalic capsule, Male spicules short and stout; gubernaculum small and complex; pre-cloacal supplement replaced by a file of stout, short setae. Marine (SMOL & COOMANS, 2006). Type and only one species: *A. nudum* Inglis, 1964 (Table 16).

Genus *Oxyonchus* Filipjev (1927)

When Filipjev (1927) created the genus *Oxyonchus* he transferred *Enoplolaimus acantholaimus* Saveljev, 1912, *E. australis* de Man, 1904, *E. dentatus* Ditlevsen, 1918 and *E. hamatus* Steiner, 1916 to the new genus. *Oxyonchus* is distinguished from other Thoracostomopsidae, including *Enoplolaimus*, by the possession of three unequal teeth (*onchia*). Two subventral teeth are very large, triangular, and outwardly curved. The dorsal tooth (*onchium*) is much smaller and knob-like. The subventral teeth, project into the buccal cavity nearly as far as, or only slightly beyond, the mandibles, which are well developed. The cephalic organ consists of paired flexible sensory structures, cirri, that project anteriorly from the external surface of the ventrolateral lips (also found in *Parasaveljevia* (WIESER, 1953) but in this genus the mandibles are reduced). A single ventral precloacal sensory supplementary organ is found in males that is characteristic of Thoracostomopsidae and Enoploidea (NICHOLAS, 2004). It was found 14 species described in the literature (Table 17). Type species: *O. hamatus* (Steiner, 1916) Filipjev, 1927. Nicholas (2004) made a key to the genus *Oxyonchus* that is modified from that given by Keppner (1988) to include subsequently described species.

1. Cuticle with transverse striations; spicules not arcuate; gubernaculum without apophysis……..……..…… *O. striatus* Keppner, 1988
Cuticle without striations; spicules arcuate; gubernaculums with or without apophysis ……..……. 2

2. Tail 9–10 cloacal diameters long…….……..*O. dubius* (Filipjev, 1919) De Coninck & Schuurmans Stekhoven, 1933.

Tail 8 cloacal diameters long or less……..…..…….....…….……... 3

3. Inner cephalic setae 0.8 head diameters or less in length…….....……..4
Inner cephalic setae 1 head diameter or more in length…….....…….....8

4. Precloacal supplement absent in male……...……...……...5
Precloacal supplement present in male……...……...……..6

5. Tail 7 cloacal diameters long; spicules 1.9 cloacal diameters long; gubernaculum with apophysis……..……...….....*O.problematicus* Filipjev, 1946
Tail 4.2 cloacal diameters long; spicules 1 cloacal diameter long; gubernaculum without apophysis……..…….....….*O. pachylabiatus* Schuurmans Stekhoven, 1946

6. Mandibles with 14–15 denticles; cephalic capsule 45–50 μm long.....*O. acantholaimus* (Saveljev, 1912) Filipjev, 1927
Mandibles with about 6 denticles; cephalic capsule less than 45 μm long ……..…….7

7. Spicules 1.5 cloacal diameters long ……..….*O. hamatus* (Steiner, 1916) Filipjev, 1927
Spicules 1.0 cloacal diameters long……..… *O. australis* (de Man, 1904) Filipjev, 1927

8. Inner cephalic setae more than 1.5 times head width…..……9
Inner cephalic setae less than 1.5 times head width……...……11

9. Spicules slender, uniformly curved, small capitulum…...…..*O. culcitatus* Wieser, 1959
Spicules stout, ‘comma-shaped’……..…….……..10

10. Mandibles low, arched, with more than 20 denticles in several rows on lower half …….....……..…...*O. longisetosus* n. sp.
Mandibles high, nearly rectangular, with up to 20 uniformly distributed denticles…… *O. evelynae* n. sp.

11. Tail length 4–4.5 times body width at cloacal opening……...…….12
Tail length 5–6 times body width at cloacal opening……....……..13

12. Mandibles with 15–20 denticles; male precloacal supplement 2–2.5 cloacal diameters from cloacal opening ….....……*O. dentatus* (Ditlevsen, 1918) Filipjev, 1927
Mandibles with 4 denticles; precloacal supplement 3.8–4.2 cloacal diameters from cloacal opening ……....……..…….*O. polaris* Filipjev, 1927

13. Precloacal supplementary organ about 2.2 cloacal diameters fom cloaca; gubernacular hypophysis directed caudally…….…..….. *O. ditlevseni* Inglis, 1964
Precloacal supplement about 3.7 cloacal diameters from cloaca; gubernacular hypophysis directed dorsally…….....……. *O. subantarcticus* Mawson, 1958

Genus *Parasaveljevia* Wieser, 1953 = ? *Hyptiolaimus* Cobb 1930b

Lips high, All anterior sensilla setose, Similar to Saveljevia but with well-developed mandibles; dorsal tooth small or reduced, two large ventro-sublateral teeth surpassing anterior

end of mandibles Cirri-shaped cephalic organs present or absent. Denticles on mandibular plates present or absent. Marine (SMOL & COOMANS, 2006).

Wieser (1953) described two *Parasaveljevia* species with cirrus-shaped cephalic organs and denticles on the mandibular plates, stating that the presence of cirrus-shaped cephalic organs and denticles on the mandibular plate point towards a close relationship with *Oxyonchus*, This renders the distinction between the two genera unclear. Three species is described in the literature (Table 18). Type species *P. clavicauda* (Filipjev, 1925).

This genus contains all species of the original genus *Saveljevia* with the mandibles well developed; it is perhaps identical with *Hyptiolaimus* Cobb 1930, but since certainty in this question can not be reached, the name should not be retained. Coob, assumes a relationship to the Oncholaimidae which view, however, is contradicted by the figure which clearly shows that *Hyptiolaimus cephalatus* belongs to the Enoplidae. It can not be decided, however, whether the genus is identical with *Parasaveljevia*, *Saveljevia* or *Oxyonchus*. It is not improbable that a still further distinction will be made within the present genus since both the new species are separated from the type species by the occurrence of “cirri” and of denticles in the mandibular fields, which points towards a closer relationship to *Oxyonchus*.

Wieser (1953) built a key for the species of this genus:

A. Cephalic setae longer than one head diameter, one “cirrus” on each lateral side of the head; mandibular fields provided with denticles:

1. Tail 9-12 anal diameters long; cephalic setae inserted at the anterior end of the head capsule:
P. lupata Wieser, 1953.

2. Tail 4,5-5 anal diameters long; cephalic setae inserted in the posterior third of the ead capsule:
P. cirrifera Wieser, 1953

B. Cephalic setae one head diameter long; no »cirri»; no denticles in the mandibular fields. *P. clavicauda* (Filipjev 1925) = *Saveljevia clavicauda*.

Geographic distribution of the valid species of the Family Thoracostomopsidae

The knowledge abouth biogeographical distribution of the nematodes is fragmented due absence of data in several regions. However, different habitats were recently explored by

Vanreusel *et al.* (2010), but according to the same authors, the geographical coverage is still not fully established.

In general, the biogeographical distribution of small deep sea endobenthic species is poorly known (GAGE, 1996; REX, 1997). Nematodes, the most abundant and probably most species endobenthic metazoan group have not yet been investigated. Nevertheless, speculations on the total number of deep sea nematodes species are on going since the discussion on the conservation of the marine ecosystem has gained more attention in recent decades (LAMBSHEAD, 1993; SNELGROVE & SMITH, 2002; LAMBSHEAD *et al.*, 2003).

Deep-sea nematode genera are well known to be cosmopolitan. For instance, Vanhove *et al.* (1999) describe nematode communities from transect towards the deep- Antarctic Sea sharing many similarities in terms of generic composition with communities identified along a Mediterranean slope transect. The apparent homogeneity of the silty sediment (VANHOVE *et al*., 1999) and the absence of marked dispersal barriers has been one of the proposed explanations for such similarities through out the large deep-sea environment (LAMBSHEAD & BOUCHER, 2003).

Based on these observations at the generic level plus the remarkable local number of species, speculations on regional to global diversity have being discussed by several authors (GRASSLE & MACIOLEK, 1992; LAMBSHEAD & BOUCHER, 2003).

For the Family Thoracostomopsidae, only two recorders found it was from Nicholas & Marples (1995) that effected a study about the geographical distribution and morphometrical of the specimes of *Enoploides stewari* Nicholas 1993, however not found data in literature for use like teorical referential for this family. Another paper of Nicholas (2007) from a sandy beach in southeastern Australia, with a key to species and the author wrote about geographical distribution

Based in the data got for this revision, it was possible observed that the species which presents a long distribution of the world like in marine environmental, deep sea, estuary, etc.

The data below shows a revision of geographical genera distribution of the Family Thoracostomopsidae (Figure 9).

Saveljevia → Northern England and Northern Ireland.

Epacanthion → Southern Brazil, Southern Ocean in the Antarctic region, Southern Philippines, South Pacific Ocean, North Hudson Bay, Northern Greenland, Northern England, Northern Ireland, North Sea, Ionian Sea, Mediterranean Sea off the coast of Italy, English Channel and South Australia.

Enoploides → Brazilian coast, Gulf of México, coast of the Equator in the Pacific Ocean, Islas Malvinas, North Atlantic Ocean off the coast of New Scotia, Southern Ocean in the Antarctic region, French Southern & Antartctic Lands, Black Sea, Adriatic Sea, Ionian Sea, Mediterranean Sea off the coast of Italy, Bay of Biscay, Northern England, Northern Ireland, North Sea, Baltic Sea and South Australia.

Mesacanthoides → Brazilian coast, Southern Pacific Ocean in Chile coast, Gulf of México, Southern Ocean in the Antarctic region, Northern England, Northern Ireland, North Sea and English Channel.

Filipjevia → Caribbean Sea, Northern Ireland, Mediterranean Sea off the coast of Italy.

Fleuronema → South Australia

Cryptenoplus → North Sea.

Fenestrolaimus → Northern England, Northern Ireland, Brazilian coast, North Sea, Southern Ocean in the Antarctic region, Florida coast, Hudson Bay, Mediterranean Sea off the coast of France and Italy.

Okranema → South Australia

Paramesacanthion → Southern Atlantic Ocean off the coast of Argentina, Brazilian coast, coast of the Equator in the Pacific Ocean, California coast, Florida coast, southern Ocean in

the Antarctic region, Northern Ireland, Northern England, North Sea, Svalbard, Northern Russia, Mediterranean Sea and Gulf of Lions, Ionian Sea and Adriatic Sea.

Mesacanthion → **N**orthern Greenland, Svalbard, Brazilian coast ,Northern England, Northern Ireland,Southern Ireland, North Sea, Southern Ocean in the Antarctic region, Gulf of México, Florida and Georgia coast, Bay of Biscay, Tyrrhenian Sea, Adriatic Sea, Black Sea, Mediterranean Sea off the coast of Italy, North Russia, Philippine Sea, Ionian Sea, Beaufort Sea, Caribbean Sea and English Channel.

Enoplolaimus → Indian Ocean off the coast of Somalia, Brazilian coast, Southern Ocean in the Antarctic region, Northern England, Northern Ireland, North Sea, Gulf of México, Mediterranean Sea off the coast of Italy and Greece, North Atlantic Ocean off the coast of New Scotia, Northern Russia, Svalbard, South Georgia and the south Sandwich Is and Ionian Sea.

Oxyonchus → Southern Ocean in the Antarctic region, Gulf of México, Mediterranean Sea, Northern Russia, Indian Ocean in Western Africa, Black Sea Ionian Sea.

Parasaveljevia → Southern Atlantic Ocean off the coast of Argentina and Northern Ireland.

Trileptium → NE Atlantic, Mediterranean, South Pacifc, Brazilian coast, North Pacifc, South Atlantic, NW Atlantic, Carribean.

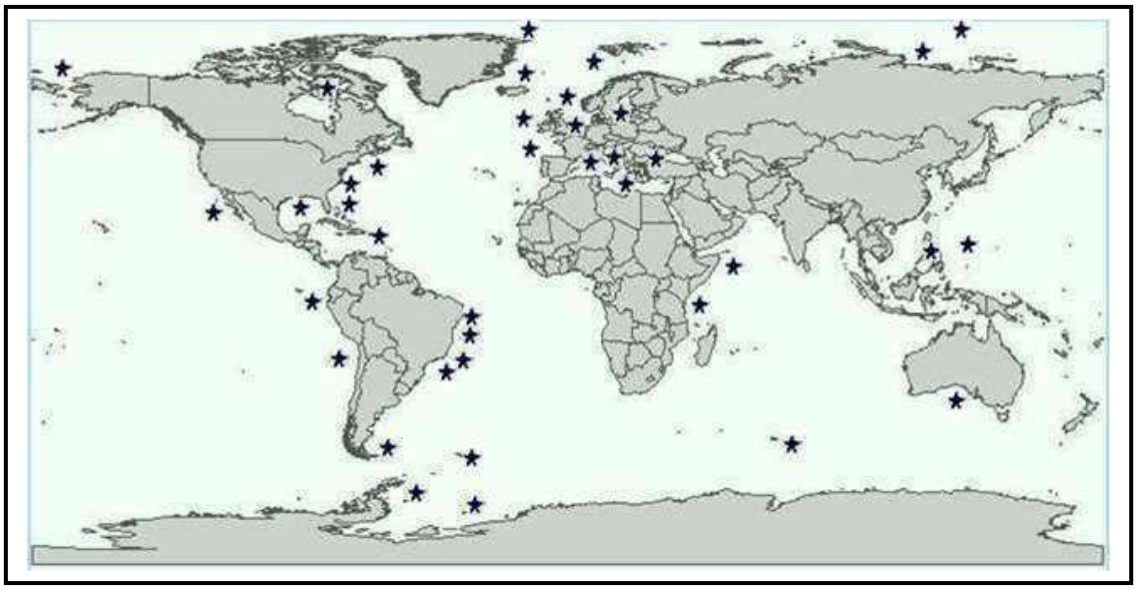


Figure 9: Geographical distributions of genus from the Family Thoracostomopsidae.

With the contents of this study, it was observed that the genera of the subfamily Enoplolaiminae: *Enoplolaimus* (21 genera), *Enoploides* (8 genera) and *Mesacanthion* (7 genera) doesn't have enough informations in the literature, even for descriptions end ilustrations. For the genera *Epacanthion* and *Oxyonchus*, the specific literature presented important and actual data about identification of valid species. The diagnosis of the family Thoracostomopsidae presents descriptions in disagree among the valid species within *Metenoploides*, *Parenoplus* and *Africantium*, however, until this moment it were not found records about geographic distribution.

Table 2: Comparative table among the species of *Saveljevia* (Drawings copied from the original papers)

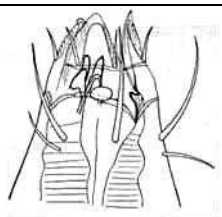
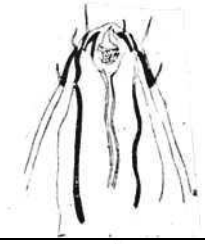
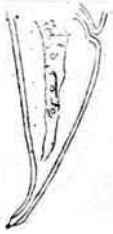
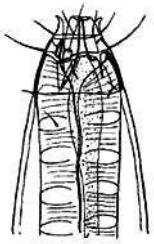
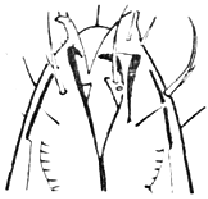
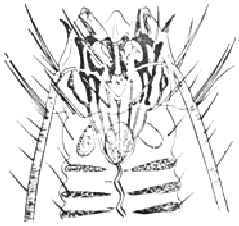
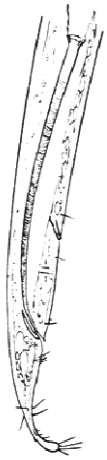
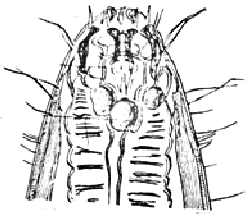
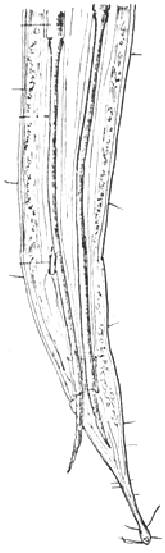
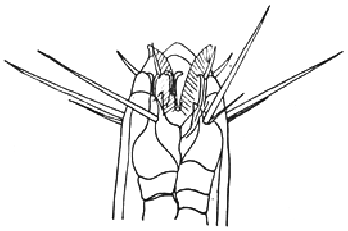
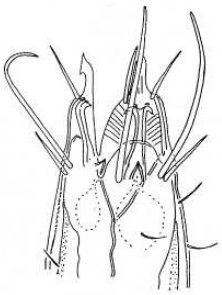
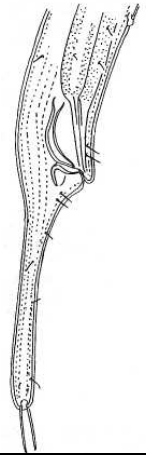
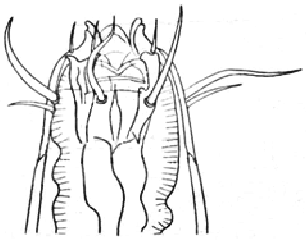
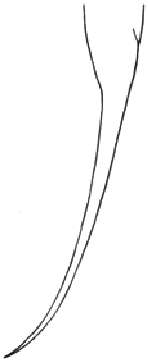
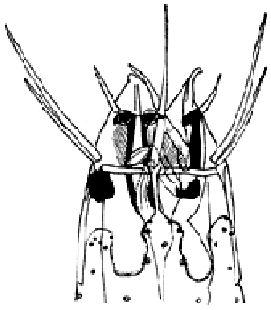
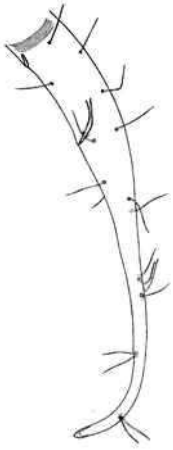
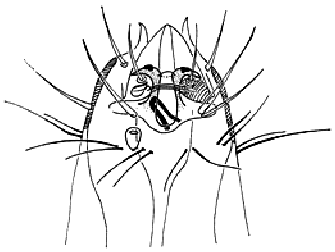
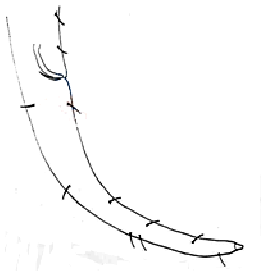
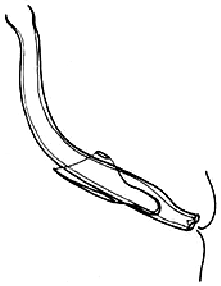
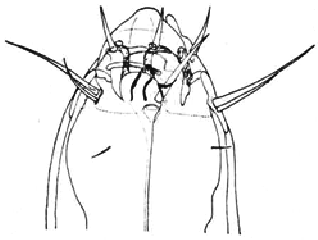
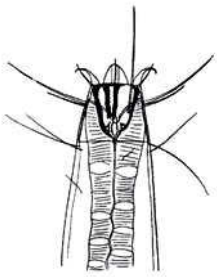
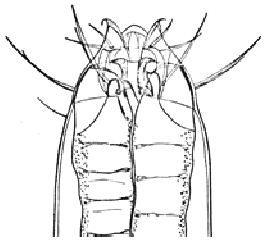
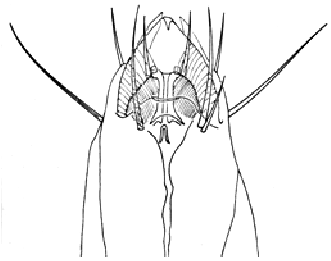
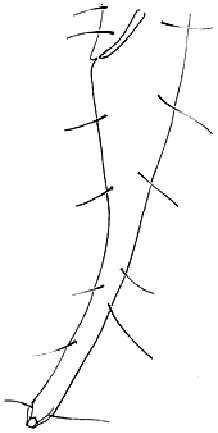
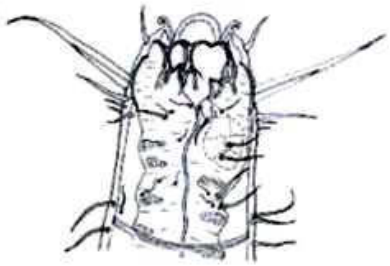
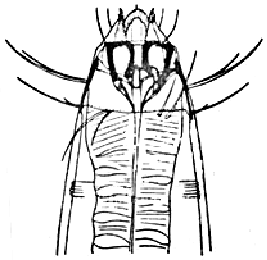
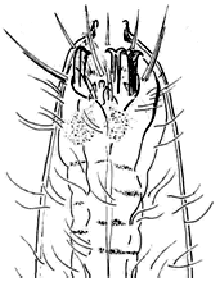
Species	Head	Spicule apparatus	Main features
Saveljevia cornuta Gerlach, 1956			Description based in females.
Saveljevia spissignatha Allgén, 1940			Description based in females.
Saveljevia hastata Wieser, 1958			Description based in females.

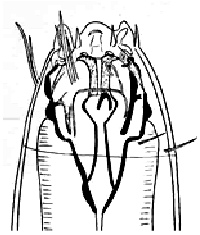
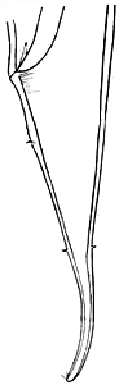
Table 3: Comparative table among the species of *Epacanthion* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
E. brevispiculum Mawson, 1956			Short spicules and enlarged proximal end. Gubernaculum absent.
E. buetschli (Southern, 1914)			Long and slender, coarsely striated, especially in distal portion, which is pointed. The proximal end is funnel-shaped. Gubernaculum short.
E. durapelle (Kreis, 1929)			Long spicules. Short gubernaculum.

E. enoploidiforme (Gerlach, 1953)			Short spicules. Simple gubernaculum.
E. exploratoris Greenslade & Nicholas, 1991			Short spicules. Gubernaculum with apophysis.
E. flagellicaudum Gerlach, 1956			Only juveniles
E. galeatum Boucher, 1977			Simple spicules. Gubernaculum with two tubular lateral pieces.

E. georgei Inglis, 1971			Simple spicules and gubernaculum.
E.gorgonocephalum Warwick, 1970			Spicules short, slightly curved. Gubernaculum with a short double tube.
E. mawsoni Warwick, 1977			Spicules arcuate. Gubernaculum a triangular plate.
E. microdentatum Wieser, 1953			Spicules 2' 5 anal diameters (80 µm)
E. multipapillatum (Wieser, 1959)			Spicules proximally cephaleted. Gubernaculum with dorsal apophysis.

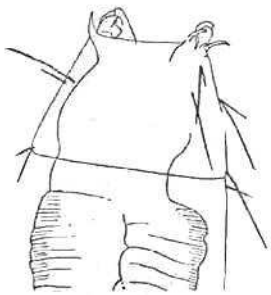
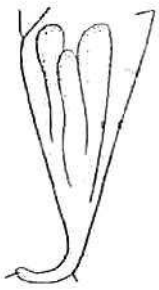
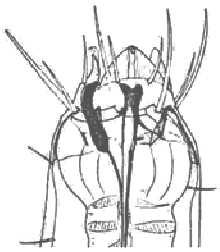
E. oliffi Inglis, 1966			Simple spicules. Gubernaculum absent.
E. oweni Keppner, 1986			Cuticle thick, smooth Spicula long With slight curve through middle ¾ of length and a recurved, broad cupshaped distal end
E. pellucidum (Ssaweljev, 1912)			Simple spicule. Gubernaculum apparently reduced.
E. polysetosum (Jensen, 1986) comb. nov.			Spicules almost straight, slightly curved at distal end. Gubernaculum slightly curved, distally with two teeth.

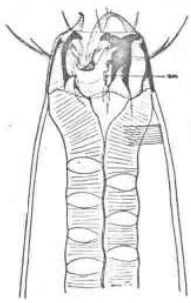
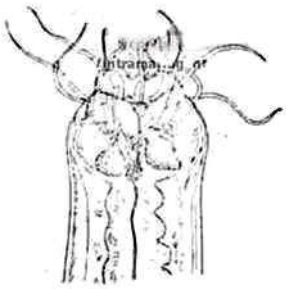
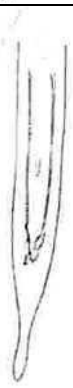
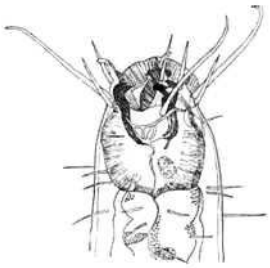
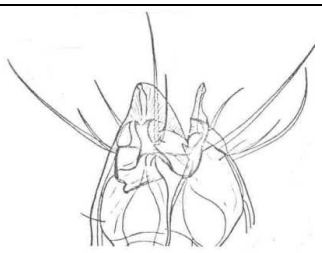
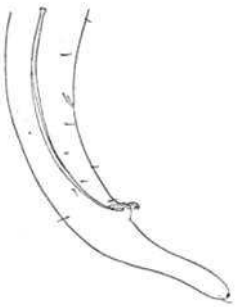
E. stekhoveni **nom. nov.** for *incurvatus* Ditlevsen, 1926 *sensu* Stekhoven, 1946			Long spicules. Simple gubernaculum.

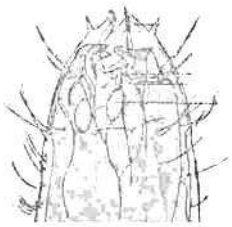
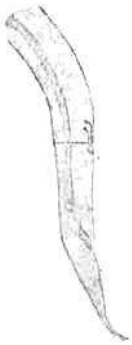
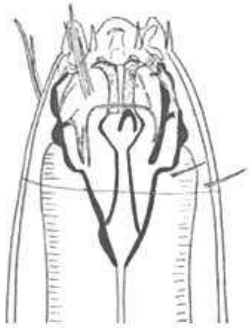
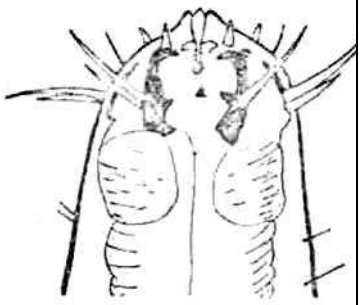
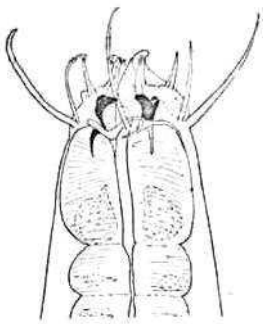
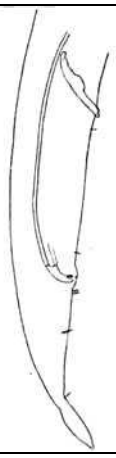
Were not gotten any information of the following species:

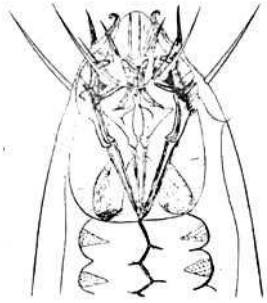
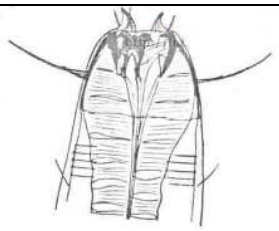
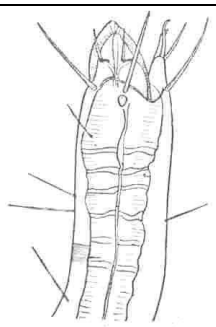
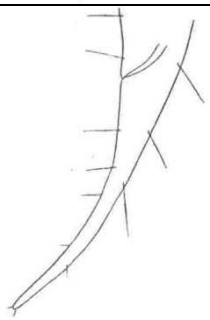
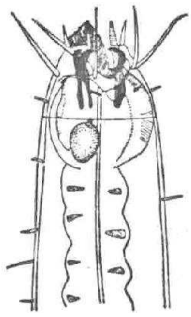
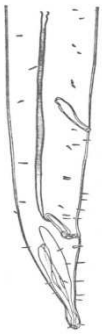
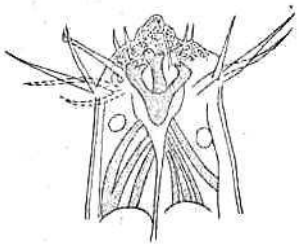
- *E. brevispiculosum* Mawson, 1958
- *E. murmanicum* (Ssaweljev, 1912)
- *E. nadjae* Sergeeva, 1974
- *E. saveljevi* (Filipjev, 1927)

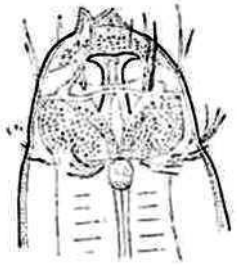
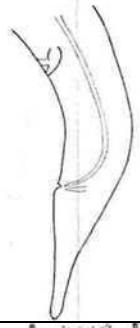
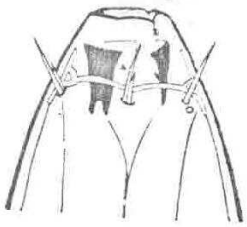
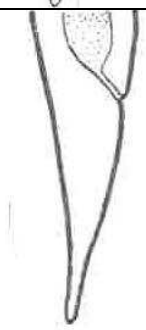
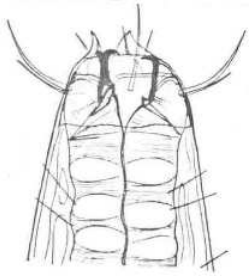
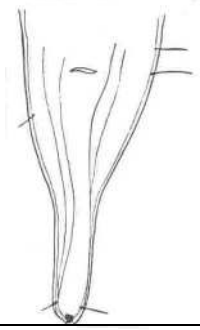
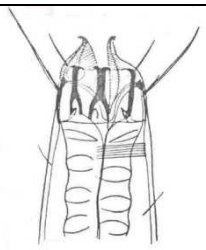
Table 4: Comparative table among the species of *Enoploides* (Drawings copied from the original papers).

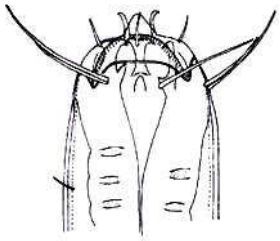
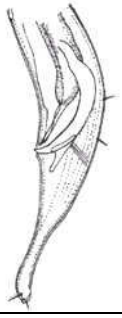
Species	Head	Spicule apparatus	Main features
E. amphioxi Stekhoven & Schuurmans, 1950			Description based in females.
E. bisulcus Wieser & Hopper, 1967			Spicula:verticall y striated throughout, with a diagonal break in ther distal end that runs from dorsal to ventral, tip pointed

E. brattstromi Wieser, 1953			Cuticle striated. Tail Description based in females.
E. cephalophorus Stekhoven & Schuumans, 1935			Spicula are exceedingly long and slender, they are somewhat expanded in the proximal end. There are two accessory pieces each of which encloses the distal end of spicula
E. delamarei			Cuticle slightly striated Spicules of the male very long, of equal size or 10-19.5 cloacal microns in diameter, with the striatum cylindrical, with a longitudinal ridge. Slightly flared and the distal end in a hook
E. harpax Wieser,1959			Spicules the spicule glide in a complicated gurbernaculum that distaly is provided with 2 hooks. Supplement tubular, simple.

E. hirsutus Filipjev,1918-1921			Spicules Spicula much longer than tail
E. incurvatus Schuumans, 1946			Cuticle clear thin Spicules of 8.1µm in length slightly striated in the proximal region and pointed at the distal end.
E. kerguelensis Mawson, 1958			Tail: Description based in females.
E. labiatus Stekhoven & Schuumans, 1935			Cuticle in the deeper layer of grease proof finely transversely striped. Spicules right spicules slightly longer than left.

E. labrostriatus Southern, 1914			Spicules very long and smooth.
E. longicaudatus Wieser, 1953			Cuticle striated. Description based in females.
E longisetosus Stekhoven & Schuumans, 1943			Description based in females.
E.longispiculosus Vitiello, 1967			Cuticle finely striated The spicules of the male, almost straight, very long and the walls of the spicula are connected by small transverse striations
E. macrochaetus Allgen, 1929			Description based in females.

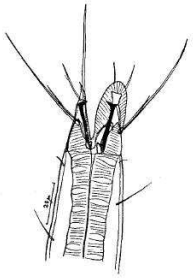
E. mandibularis Coles, 1977			The cuticle is transversely striated**.** The spicules are long, of simple form and about one and a third as long as the length of the tail
E. oligochaetus Mawson, 1955			The cuticle is irregulary pitted Tail: Description based in females.
E. paralabiatus Wieser, 1953			Description based in females.
E. polysetosus Jensen, 1986			Cuticle smooth Spicules almost straight, slightly curved at distal end; cut cuticularization with a granulated appearance
E. reductus Wieser, 1953			Description based in females.

E. sabulicola Allgen, 1933			Description based in females.
E. stewarti Nicholas, 1993			Cuticle without annulations. Spicules uniformly curved, cyathiform, with proximal cylindrical knob

Were not gotten any information of the following species:

- *E. cirrhatus* Filipjev, 1918
- *E.crassus* Greenslade & Nicholas, 1991
- *E. filicaudatum* Greenslade & Nicholas, 1991
- *E. gryphus* Wieser & Hoppe. 1967
- *E. spiculohamatus* Schulz, 1932
- *E. tyrrhenicus* Gerlach ,1953
- *E. uniformis* not information
- *E. vectis* Gerlach , 1957

Table 5: Comparative table among the species of *Metenoploides* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
M. alatus Wieser, 1958			Description based in females.

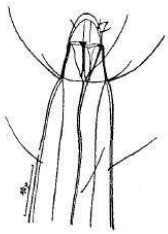
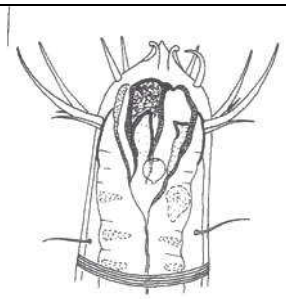
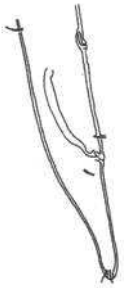
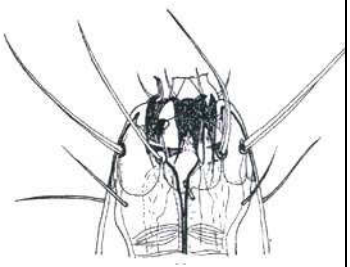
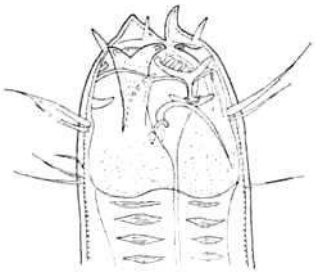
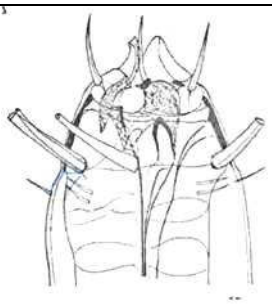
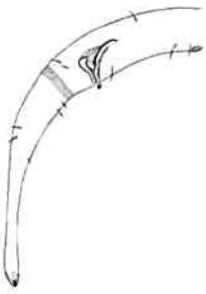
M. capitulum Wieser, 1958			Description based in females.

Table 6: Comparative table among the species of *Mesacanthoides* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
M. brevicaudatus Keppner, 1987			Cuticle thin with very fine transverse striations most evident anteriorly and in anal region. Spicules 54 (52-56) long, with gently curved middle portion and acutely curved, forked tip.
M. fibultus Wieser & Hopper, 1967			Spicules more than two anal diameters long, tail filiform, with flagellum
M. magna Coles, 1977			Description based in females.
M. psittacus Wieser & Hopper, 1967			Spicules nearly straight, tip elaborately armed

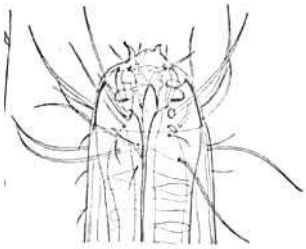
M. sinuosus Wieser, 1959			Spicules strongly arcuate, tip pointed
M. wieseri Mawson, 1956			Description based in females.

Table 7: Comparative table among the species of *Filipjevia* (Drawings copied from the original papers).

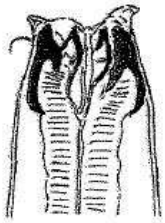
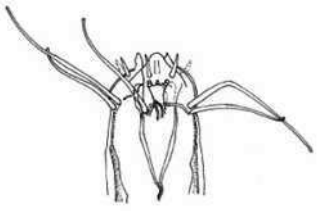
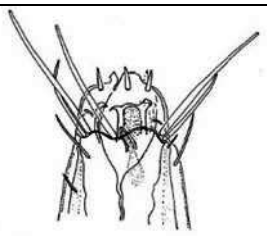
Species	Head	Spicule apparatus	Main features
F. macrolabiata Kreis, 1928			Were not gotten any information

Table 8: Comparative table among the species of *Fleuronema.* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
F. dorca Greenslde & Nicholas, 1991			Cuticles thick and smooth. Spicules heavy and encircled by a distinct ridge which divides spicules in half
F. robusta Greenslde & Nicholas, 1991			Spicules with thick wall, but long and thin, with hooked tip, 1 6 x anal.

Were not gotten any information of the following species:

- *F. tridentatus (Ssaweljev) comb. Nov.* Greenslde & Nicholas, 1991 = *Enoploides tridentatus Ssaweljev, 1912: 116.*Table 9: Comparative table among the species of *Parenoplus*

Table 9: Comparative table among the species of *Parenoplus* (Drawings copied from the original papers).

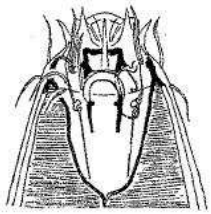
Species	Head	Spicule apparatus	Main features
P. edentatus Filipjev, 1927			Were not gotten any information

Table 10: Comparative table among the species of *Cryptenoplus* (Drawings copied from the original papers).

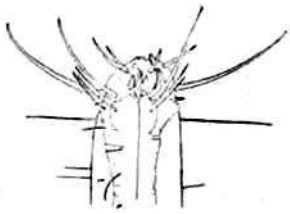
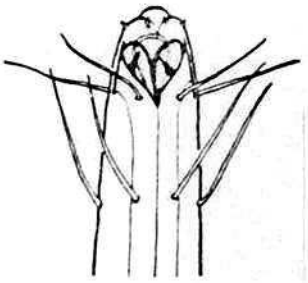
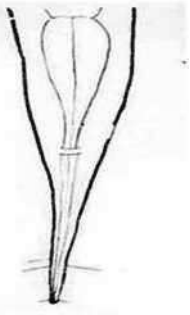
Species	Head	Spicule apparatus	Main features
C. gerlachi Riemann, 1964			Were not gotten any information

Table 11: Comparative table among the species of *Fenestrolaimus* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
F. antarticus Mawson, 1956			Description based in females.

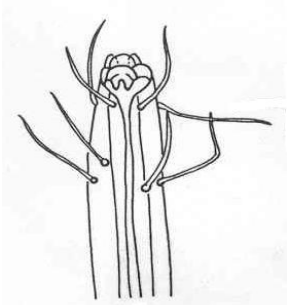
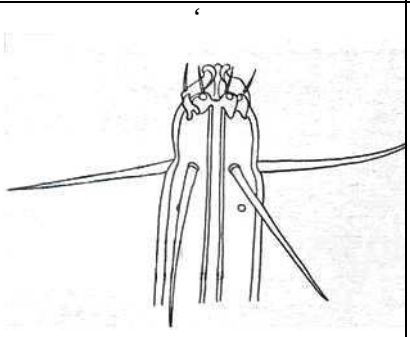
F. genus Vittielo,1970			Description based in females.
F. vestitus Gerlach, 1956			Description based in females.

Table 12: Comparative table among the species of *Okronema*(Drawings copied from the original papers).

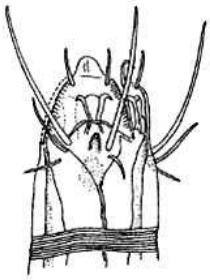
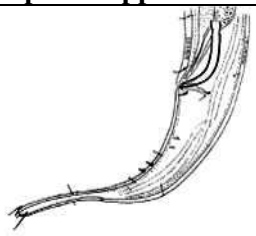
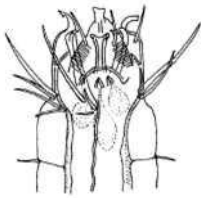
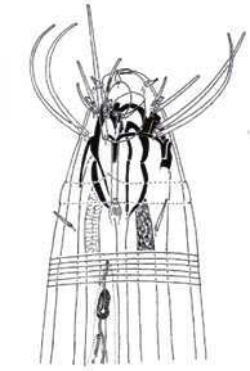
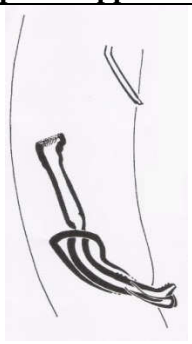
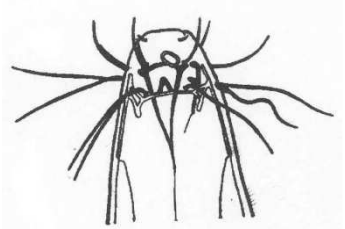
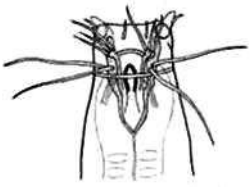
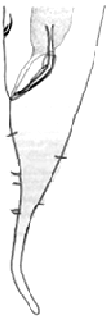
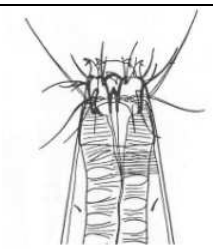
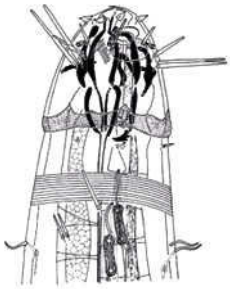
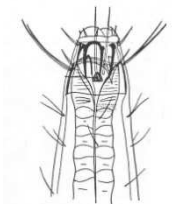
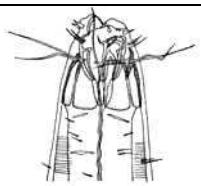
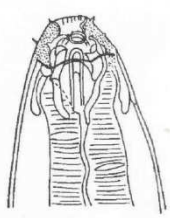
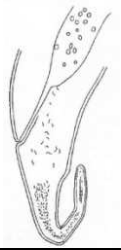
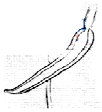
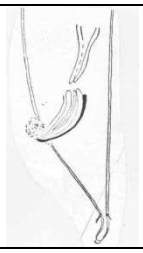
Species	Head	Spicule apparatus	Main features
O. eileenae Greenslde & Nicholas, 1991			Cuticles Lip flaps well developed, with ridges and about 20 grooves forming a striated border. **S**picule heavy and curved, top rounded, tip apparently with pair of end pegs.
O.militaris Greenslde & Nicholas, 1991			Cuticles annulated Spicules curved and heavy, 11/4X anal diameter;

Table 13: Comparative table among the species of *Paramesacanthion* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
P. abyssorum Bussau, 1995			Cuticles In the middle of the body the very weakly annulated Spicules The double-jointed spicules are 155 µm long and are divided into two halves by a sharp constriction.
P.allgeni Mawson,1958			Spicules shorter then 1-2 cloacal diameters
P. catellus Boucher, 1970			Cuticles is smooth Spicules 1.5 cloacal diameters
P. estridia Wieser, 1953			Spicules longer 2.4 cloacal diameters long, no cervical setae in groups
P.forceps Bussau, 1995			Cuticles The very weakly annulated cuticle (annule width 2 µm) is 7 µm thick Spicules The double-jointed spicules are 350 µm long.

P. hirsutum Warwick, 1970			Cuticles is marked internally by fine transverse striations. Spicules shorter then 1-2 cloacal
P. inaequale Wieser, 1953.			Spicules longer 4-5 cloacal diameters long, oesophageal region with dense groups of cervical setae
P. marei Warwick, 1970			Spicules longer then 2-3 cloacal. Not barbed, tail with only four terminal setae none at all
P. microsetosum Mawson, 1956			Description based in females.
P. oxycephalus Ditlevsen,1926			Spicules shorter then 1-2 cloacal diameters
P. paroxycephalum Allgén, 1959			Were not gotten any information

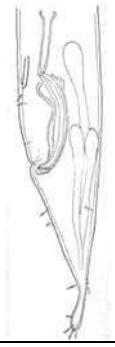
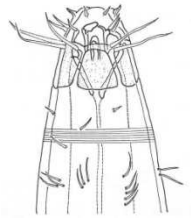
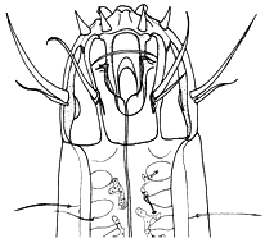
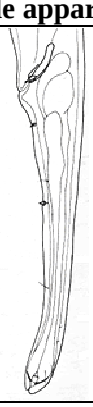
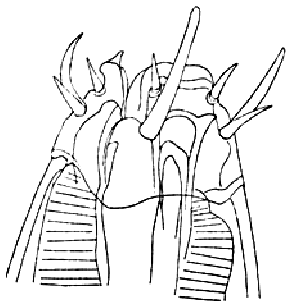
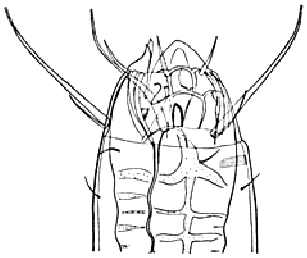
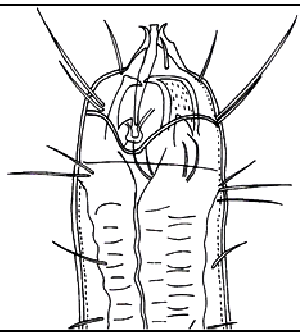
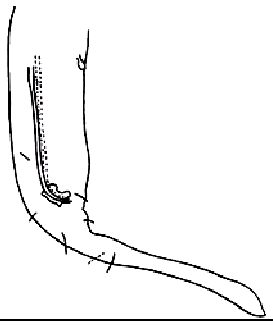
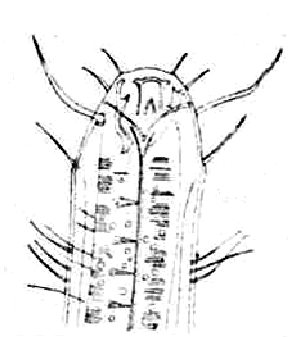
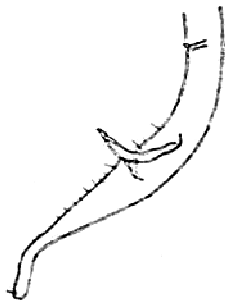
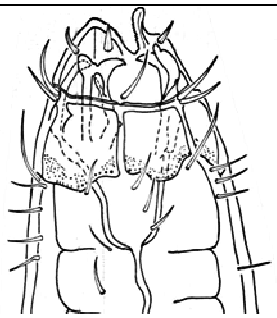
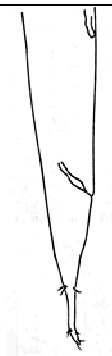
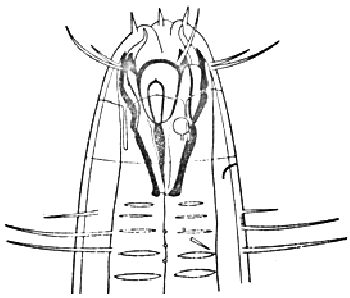
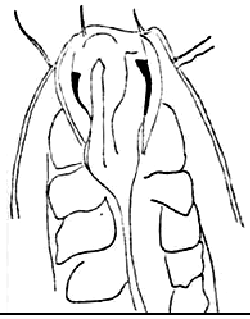
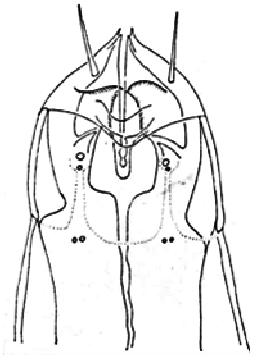
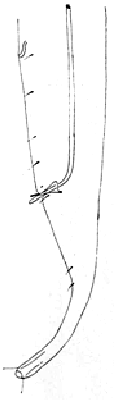
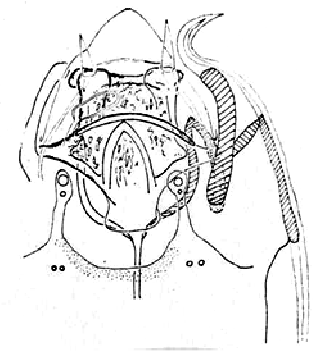
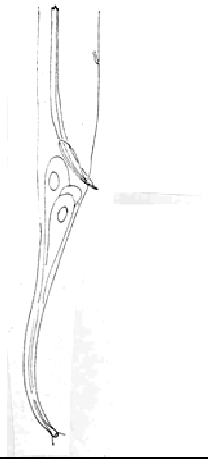
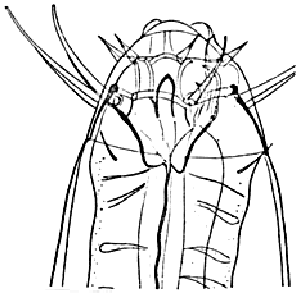
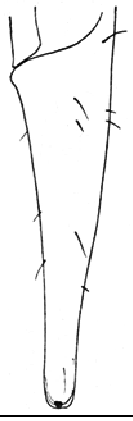
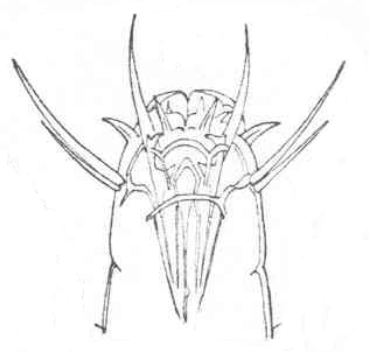
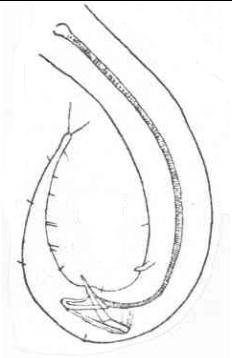
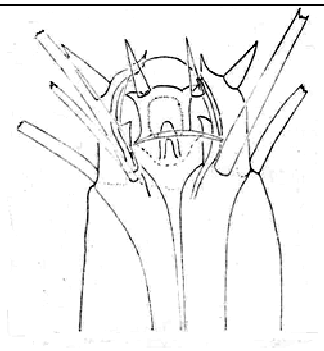
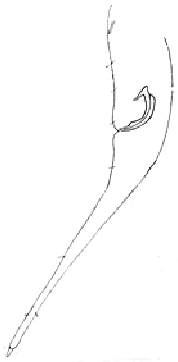
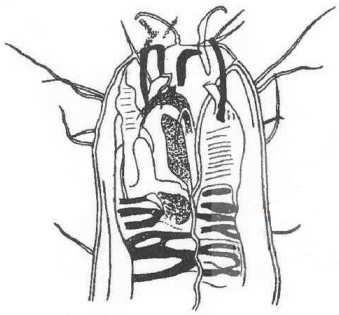
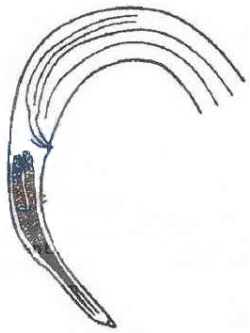
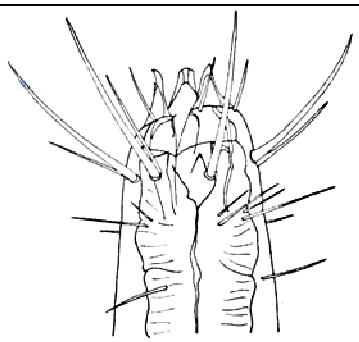
P. tricuspis Vitiello, 1970			Spicules longer, 2.5 cloacal diameters long, distal portion narrower than proximal
P. truncus Vitiello, 1971			Were not gotten any information

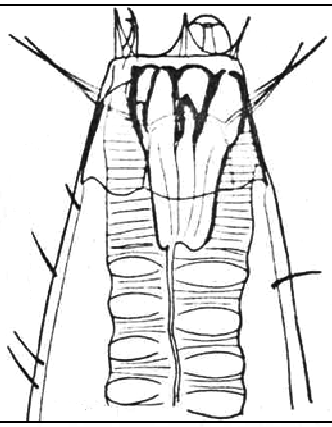
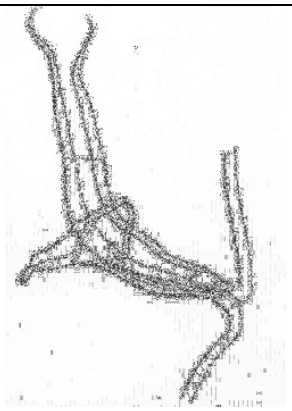
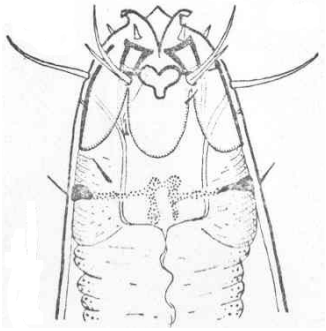
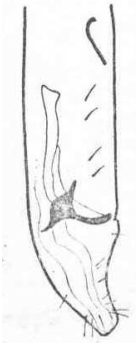
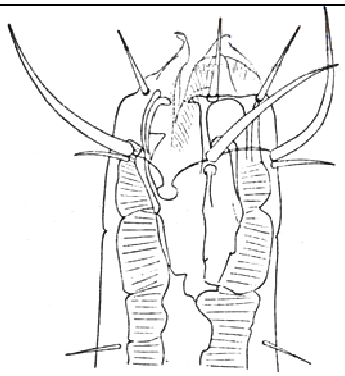
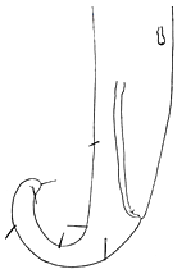
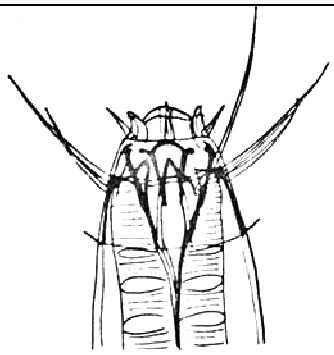
Table 14: Comparative table among the species of *Mesacanthion* (Drawings copied from the original papers).

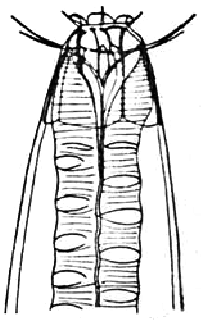
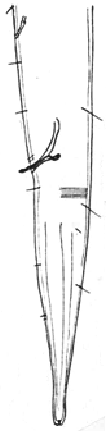
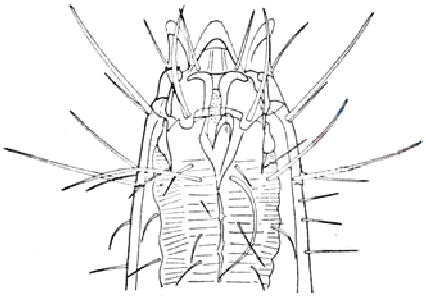
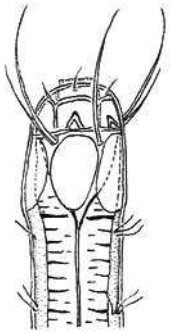
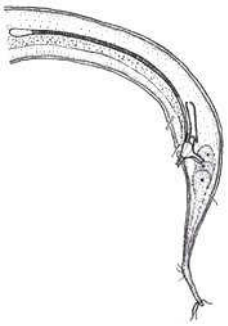
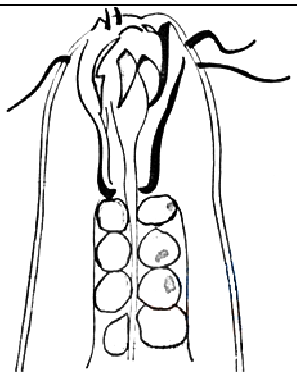
Species	Head	Spicule apparatus	Main features
M. africanthiforme Warwick, 1970			Spicules small and fairly straight. Tubular gubernaculum.
M. africanum Gerlach, 1957			Spicules strongly sclerotized. Gubernaculum with dorsal apophysis.

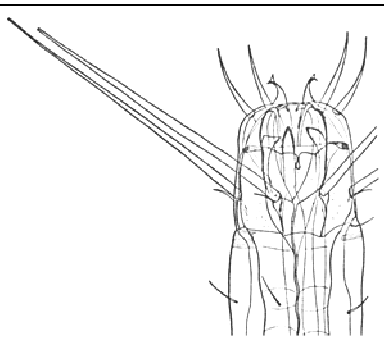
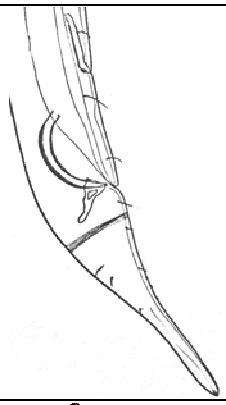
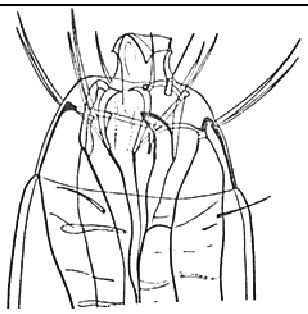
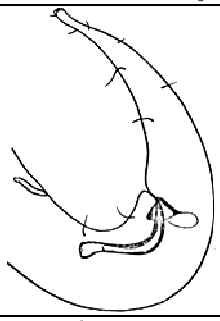
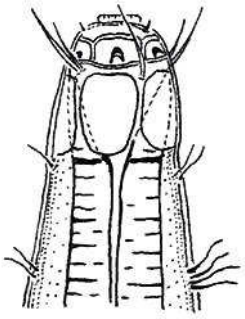
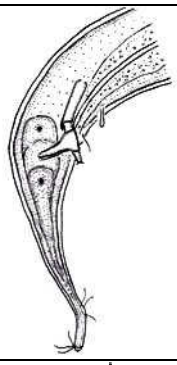
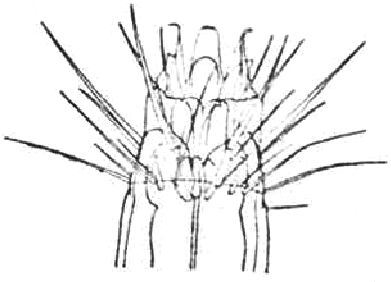
M. arcuatile Wieser, 1959			Only females described.
M.alexandrinus Nicholas, 1993			Two long slightly unequal spicules. Gubernaculum flat plate with two terminal hooks, no apophysis.
M. audax (Ditlevsen, 1918)			Spicules large and strongly curved. The tip has pointing spines. Gubernaculum with apophysis.
M. agubernatus Vitiello, 1971			Spicules arrow-like. Gubernaculum absent.
M. armatum Wieser, 1959			Spicule cephalated. Gubernaculum with two blunt terminal processes.

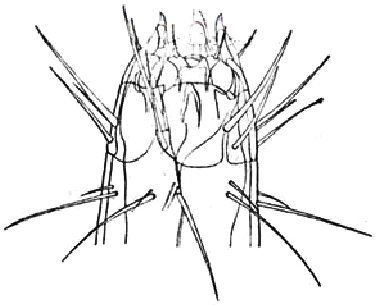
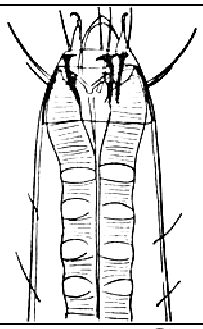
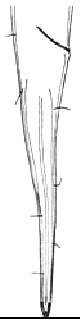
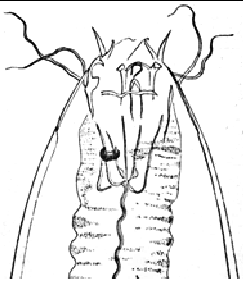
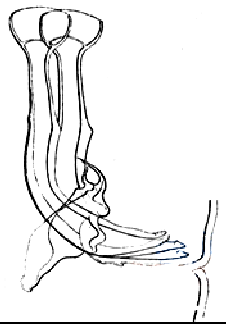
M. brachycolle Allgén 1959			Only females described.
M. cavei Inglis, 1964			Spicules equal and long. Gubernaculum short.
M. ceeum Inglis, 1964			Spicules long with distinct alae on their posterior end which stops slightly anterior to the extreme posterior tip. Gubernaculum very lightly built and clings close to the spicules.
M. cricetoides Wieser, 1959			Only females described.

M. diplechma Boucher, 1977			Spicules long and striated. Complex gubernaculum.
M. fricum Inglis, 1966			Spicules curved. Presence of the gubernaculum is uncertain.
M.gracilisetosus Allgén, 1930			Simple spicules. Gubernaculum short.
M.hirsutum Gerlach, 1953			Spicules lightly curved. Gubernaculum absent.

M. infantile Ditlevsen, 1930			Long spicules with cephalated proximal end. Gubernaculum with dorsal half expanded, extending antero-ventrally around the spicules and postero-dorsally into a spur.
M. kerguelense Mawson, 1958			Long spicules. Gubernaculum with apophysis.
M. longispiculum Gerlach, 1958			Long spicules. Gubernaculum absent.
M. longissimesetosum Wieser, 1953			Spicules short and lightly curved. Gubernaculum simple.

M. majus Wieser, 1953			Short spicules with the proximal and not dilated. Gubernaculum with apophysis.
M. monhystera Gerlach, 1967			Short spicules with cephalated proximal end. Gubernaculum absent.
M. obscurum Gagarin & Klerman, 2006			Spicules different I size and structure. Gubernaculum small, embracing both spicules, with apophysis.
M. pacificum Allgén, 1951			Spicules short, moderately slender and open at the proximal end. Gubernaculum small.

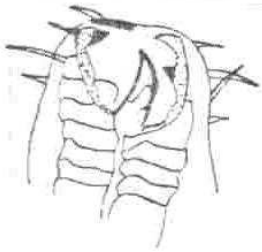
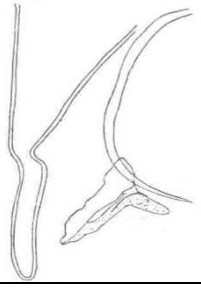
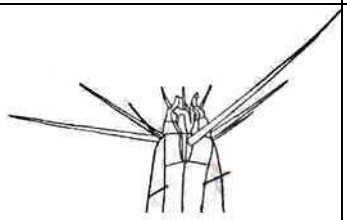
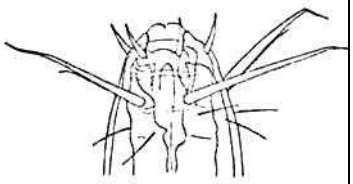
M. pali Wieser, 1959			Spicules strongly curved and sclerotized. Gubernaculum with well developed apophysis.
M. pannosum Wieser, 1959			Spicules long, cephalated proximally. Gubernaculum strong with powerful dorsal apophysis.
M. paradentatus Allgén, 1932			Only juveniles described.
M. propinquum Gagarin & Klerman, 2006			Cuticle smooth. Spicules paired, smooth, anisomorphic and anisometric.
M. proximum Gerlach, 1957			Spicules short. Gubernaculum absent.

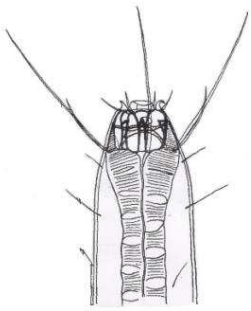
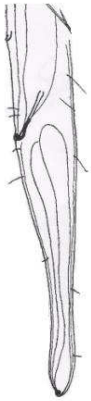
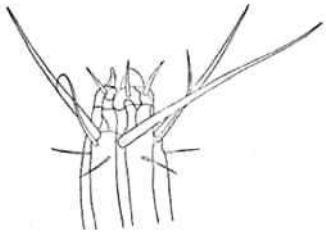
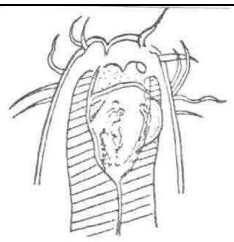
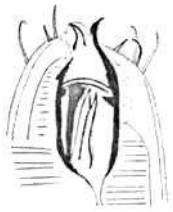
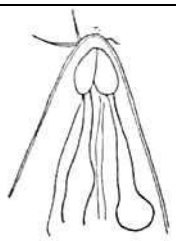
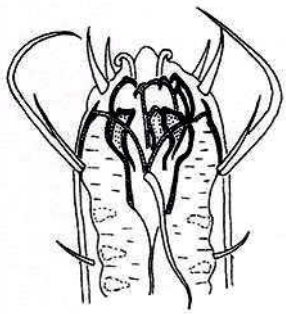
M. rigens Gerlach, 1957			Spicules strongly curved. Gubernaculum absent.
M. ungulatum Wieser, 1953			Only juveniles described.
M. virile Diltevsen, 1930			Spicules strongly curved, cephalated proximally. Gubernaculum embracing the spicules, apophysis present.

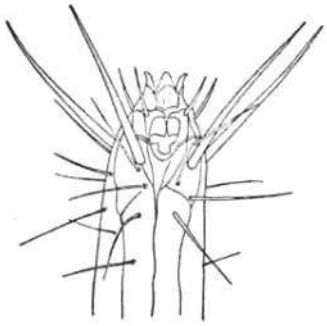
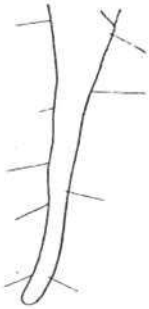
Were not gotten any information of of the following species:

- *Mesacanthion banale* Filipjev, 1927
- *M. breviseta* Filipjev, 1927
- *Mesacanthion conicum* Filipjev, 1918
- *Mesacanthion karense* (Filipjev, 1927)
- *Mesacanthion lucifer* Filipjev, 1927
- *Mesacanthion southerni* Warwick, 1973
- *Mesacanthion tenuicaudatum* Ssaweljev,1912

Table 15: Comparative table among the species of *Enoplolaimus* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
E. abnormis Kreis, 1928			Were not gotten any information
E. arcospiculum Allgén, 1959			Spicules Short strongly curved
E.attenuatus Gerlach, 1953			Spicules long 36-38 lang
E. balgensis Gerlach, 1953			Were not gotten any information

E. connexus Wieser, 1953			Spicules curved, not fused.
E. litoralis Gerlach, 1954			Spicules straight, fused in the distal half; accessory piece reduced
E. niger Allgén, 1959			Description based in females.
E. opacus Allgén, 1959			Description based in females.
E. pararegius Keppner, 1987			Cuticle thin. Spicules 42 (40-43) long, arcuate, tips curved laterally.

E. villosus Gerlach, 1953			Description based in females.

Were not gotten any information of of the following species:

- *E.cirrhatus* Allgén, 1940
- *E. denticulatus* Warwick, 1970
- *E. distortus Gerlach, 1957*
- *E. enatus* Hopper, 1962
- *E. falklandiae* Allgén 1959
- *E. filiformis* Allgén, 1959
- *E. glabrus Brunetti, 1949*
- *E. lenunculus* Wieser, 1959
- *E. longicaudatus* Southern,.1914
- *E. medius* Pauljuh, 1984
- *E. mus* Inglis, 1964
- *E. notropopinquus* Allgén, 1959
- *E. paralitoralis* Wieser, 1959
- *E. parapropinquus* Allgén, 1949
- *E. propinquus* De Coninck & Stekhoven 1933
- *E.psammae* Gerlach, 1952
- *E. punctatus* Hopper, 1961
- *E. regius* Hopper, 1961
- *E. robustus* Gerlach, 1954
- *E. subterraneus Gerlach, 1954*
- *E. zosterae Schulz, 1932*

Table 16: Comparative table among the species of *Africanthion* (Drawings copied from the original papers).

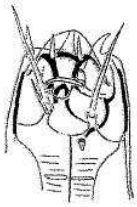
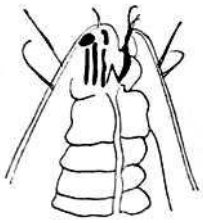
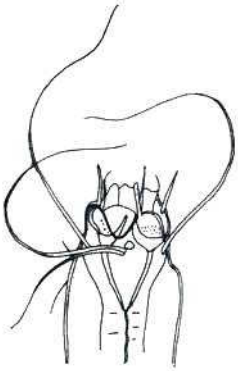
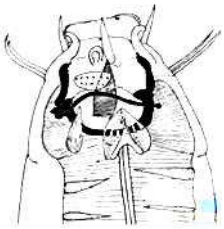
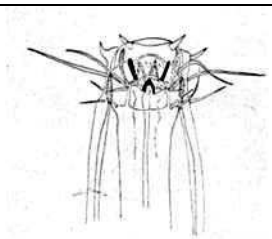
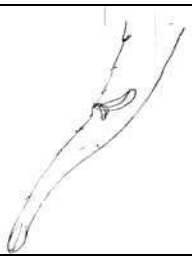
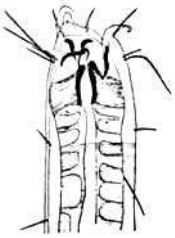
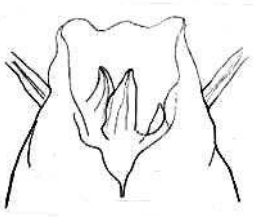
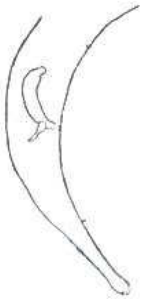
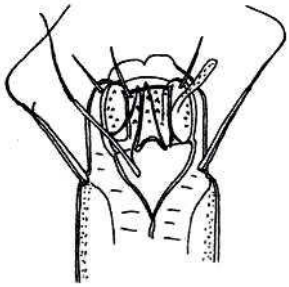
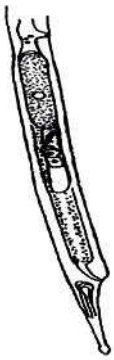
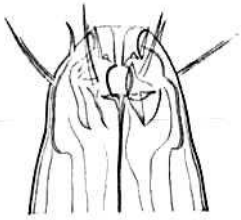
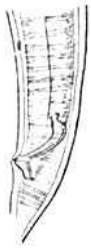
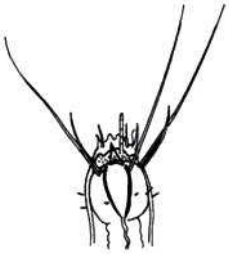
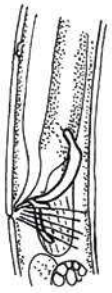
Species	Head	Spicule apparatus	Main features
A.nudan Inglis, 1964			Spicules Short and stout; gubernaculum small and complex; pre-cloacal supplement replaced by a fIle of stout, short setae.

Table 17: Comparative table among the species of *Oxyonchus* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
O. australis De Man 1904			Cuticles without striations Spicules 1.0 cloacal diameters long
O. brachysetosus Allgén, 1959			Description based in females.
O. crassicollis Allgén, 1959			Description based in females.
O. culcitatus Wieser, 1959			Cuticles without striations Spicules slender, uniformly curved, small capitulum, curved into segment of a semicircle.
O. dentatus Ditlevsen, 1919			Description based in females.
O. ditlevsen Inglis, 1964			Cuticles without striations Spicules Arcuate

O. dubius Stekhoven, 1950			Description based in females.
O. elegans Schultz, 1932			Were not gotten any information
O. evelynae Nicholas, 2004			Two spicules, stout , rounded capitula followed by narrower neck and curved shaft terminating in peg-like tips.
O. hamatus *Steiner, 1916*			Cuticles without striations Spicules 1.5 cloacal diameters long
O. longisetosus Nicholas, 2004			Spicules paired, stout, capitulum prominent, slighly narrower neck, main shaft curves through 90◦, tip peg-like.

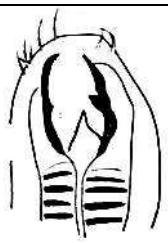
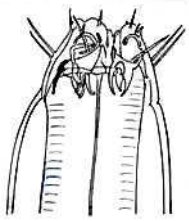
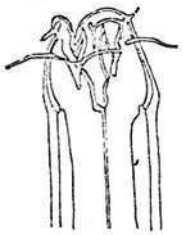
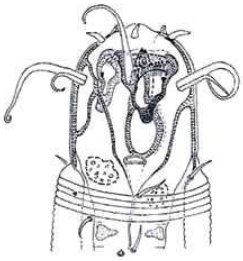
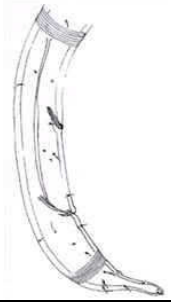
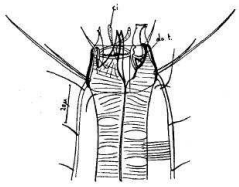
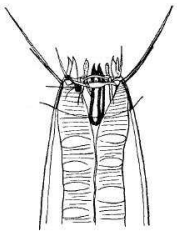
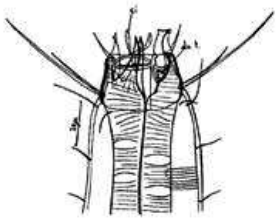
O. macrodon Allgén ,1959			Description based in females.
O. notodentatus Allgén,1959			Description based in females.
O. pachylabiatus Schuurmans Stekhoven, 1946			Cuticles without striations Spicules 1 cloacal diameter long
O. problematicus Filipjev, 1946			Cuticles without striations Spicules 1.9 cloacal diameters long
O. subantarticus Mawson, 1958			Cuticles without striations Spicules arcuate
O. striatus Keppner, 1988			Cuticles with transverse striations; Spicules not arcuate;

Table 18: Comparative table among the species of *Parasaveljevia* (Drawings copied from the original papers).

Species	Head	Spicule apparatus	Main features
P. cirrifera Wieser, 1958			Cuticles Finely striated Spicules Were not gottenany information
P. lupata Wieser, 1958			Description based in females.
P.cirrifera Wieser, 1958			Were not gotten any information

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CAPÍTULO 02

Three new species of Thoracostomopsidae Filipjev, 1927 (Nematoda) of the Atlantic Southeastern.

Three new species of Thoracostomopsidae Filipjev, 1927 (Nematoda) from the southwest Atlantic

Betânia Cristina Guilherme[1,2] Maria Cristina da Silva[1] Verônica da Fonsêca-Genevois[1] Luana Cassandra Breitenbach Barroso Coelho [3]& Maria Tereza dos Santos Correa[3]

[1] Dept. Zoologia – CCB – UFPE – Cidade Universitária - Recife – Brazil - CEP 50670-420.
[2] Universidade Federal de Campina Grande – Olho d'Água da Bica – Cuité - Paraíba – Brazil - CEP 58175-000. E-mail: betaguilherme@yahoo.com.br
[3] Dept. Bioquímica – CCB – UFPE – Cidade Universitária - Recife – Brazil - CEP 50670-420.

Abstract

The family Thoracostomopsidae from the Potiguar Basin (Rio Grande do Norte, Brazil) is composed by five genera: *Oxyonchus* Filipjev, 1927; *Trileptium* Cobb, 1933; *Fenestrolaimus* Filipjev, 1927; *Mesacanthion* Filipjev, 1927; and *Epacanthion* (Wieser, 1953). The last two genera contain new species, described here, one from *Epacanthion* (*Epacanthion canceriformis* sp. nov.) and two from *Mesacanthion* (*Mesacanthion henriques* sp. nov. and *Mesacanthion dentantuspiculum* sp. nov.).

Résumé

La famille Thoracostomopsidae du bassin Potiguar (Rio Grande do Norte, Brésil) est composée par cinq genres: *Oxyonchus* Filipjev, 1927; *Trileptium* Cobb, 1933; *Fenestrolaimus* Filipjev, 1927; *Mesacanthion* Filipjev, 1927; and *Epacanthion* (Wieser, 1953). Des deux derniers genres, trois nouvelles espèces sont ici décrites: une du genre *Epacanthion* (*Epacanthion canceriformis* sp. nov) et deux du genre *Mesacanthion* (*Mesacanthion henriques* sp. nov. and *Mesacanthion dentantuspiculum* sp. nov.).

Key-words: Nematoda, Thoracostomopsidae, *Epacanthion*, *Mesacanthion* new species

Introduction

The family Thoracostomopsidae belongs to the order Enoplida Filipjev, 1929, in possessing a basically smooth cuticle, metanemes, non-spiral amphids, and a cephalic arrangement of 6+6+4. This family is composed by three subfamilies: Thoracostomopsinae Filipjev, 1927; Trileptiinae Gerlach & Riemann, 1974; and Enoplolaiminae De Coninck

(Smol & Coomans, 2006). The features of the subfamilies are summarized by the presence or absence of the teeth, and mandibles within the buccal cavity.

The numbers of studies on the family Thoracostomopsidae in Brazil are few (Gerlach, 1956; 1957a; 1957b), but in recent years only one species was described by Guilherme *et al.*, (2009).

The present study treats material collected in the Potiguar Basin off northeast Brazil, as part of a project sponsored by PETROBRAS S. A. (the Brazilian Petroleum Company). The Petrobras project covers the the continental shelf of the Potiguar Basin, with the aim of evaluating the benthic community structure and composition, and patterns of spatial variation, at two different sampling areas/scales: Potiguar Basin (macroscale) and Guamaré Submarine Outfall (mesoscale).

Thoracostomopsidae in the Potiguar Basin is composed by five genera: *Oxyonchus*, *Trileptium*, *Fenestrolaimus*, *Mesacanthion* and *Epacanthion*. New species of the last two genera are described here.

Material & Methods

Study area and Methodology

In the Potiguar Basin (35°30′ S and 35° 37° W), 28 stations were prospected, covering depths from 1 to 71 meters (Figure 1 and 2). Depending on the depth, sediment samples were taken with a Van Veen grab or a box corer from the research vessel *Astro Garoupa*, or by diving. Sub-samples were stored in plastic containers and fixed with 4% saline formaldehyde. In the laboratory, the samples were washed through 0.5 mm mesh sieves, and the nematodes were gently picked out with a stainless-steel stylet, fixed with 4% formaldehyde, and gradually transferred to glycerin (De Grisse, 1969). Drawings were made using an OLYMPUS CX 31 optical microscope, with the aid of a drawing tube. Photos were taken with a C–5050 Zoom Olympus digital camera.

The holotypes and allotypes of all the species are deposited in the National Museum of Rio de Janeiro (MNRJ). Paratypes mounted on slides are deposited in the Nematode Collection of the Laboratory of Meiofauna (NM LMZOO), Department of Zoology, Federal University of Pernambuco, Brazil.

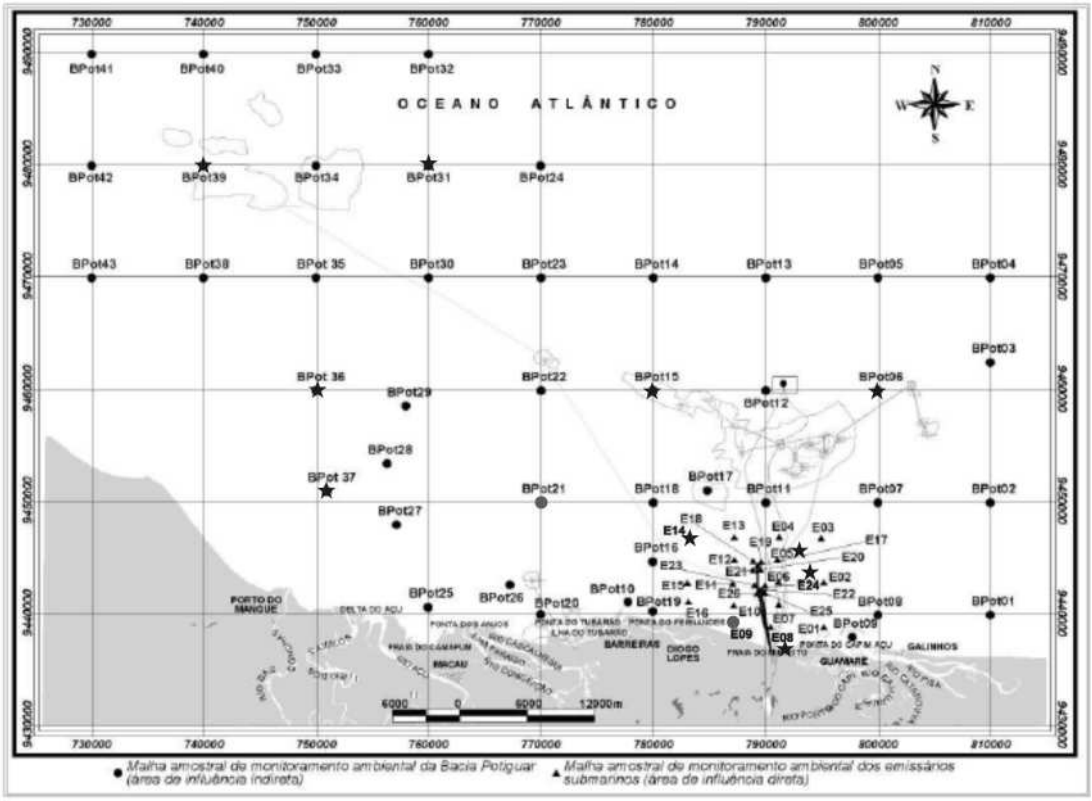

Figure 1: Localization of the stations benthic sampling stations in the Potiguar Basin during 2004.

Figure 1:Localisation des stations d´échantillonage du benthos dans la Baie Potiguar, Brésil, pendant 2004.

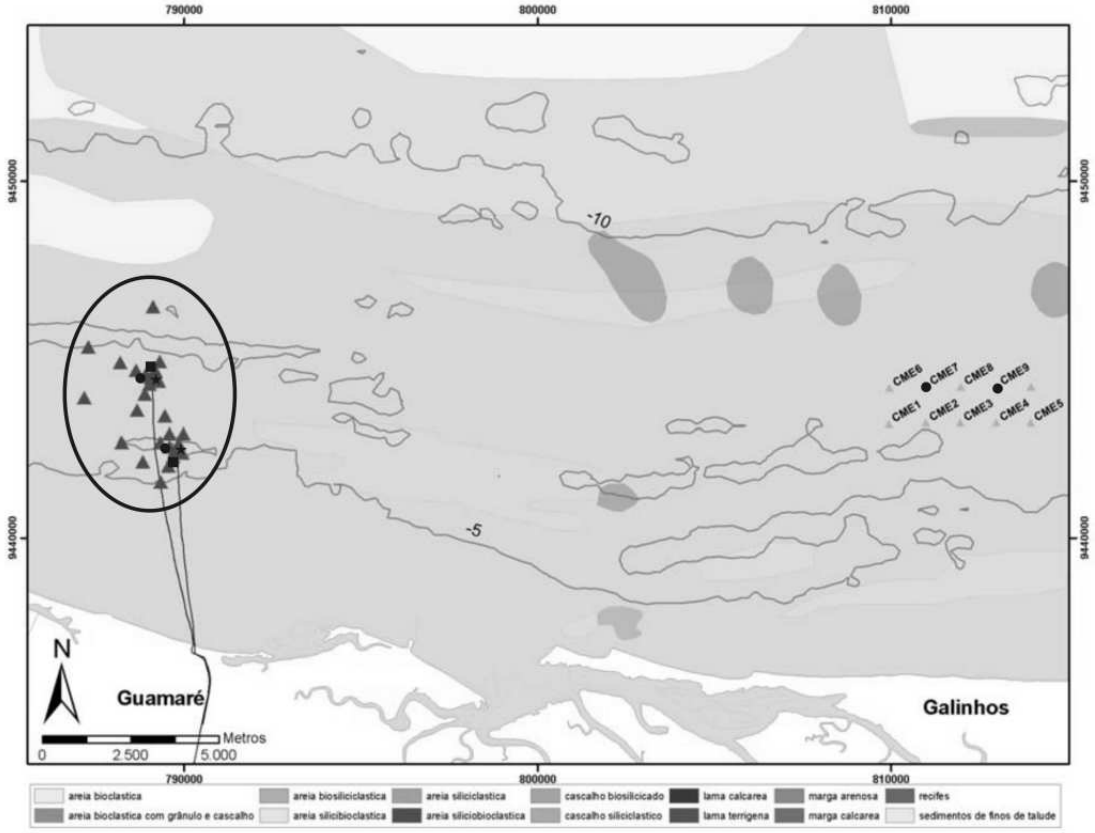

Figure 2: Localization of the stations benthic sampling stations in the Potiguar Basin during 2008.
Figure 2: Localisation des stations d´échantillonage du benthos dans la Baie Potiguar, Brésil, pendant 2008.
Abbreviations used in the text

abd: anal body diameter

amph.: diameter of amphidial fovea

Amph%: percentage of amphidial fovea diameter in relation to head diameter

amphd. pos.: distance of amphidial fovea from anterior end

hd: head diameter

cbd: corresponding body diameter

c.s.: length of cephalic setae

subc.s.: length of sub-cephalic setae

cerv.s.: length of cervical setae

gub.: length of gubernaculum

hd: head

L: body length

mbd: maximum body diameter

ph: length of pharynx

nr: position of nerve ring from anterior body end

som.s.: length of somatic setae

mab.w.: width of mandibles

mab.l.: length of mandibles

spic.: length of speculum along the arc

supl: length of supplement

t: tail length

v: distance of vulva from anterior end

V %: position of the vulva as percentage of body length from anterior end

a: L/mbd

b: L/ph

c: L/t

c': t/abd

Terms for body regions are according to Coomans (1979).

Classification (after Smol & Coomans, 2006)

Class Enoplea Inglis, 1983

Subclass Enoplia Pearse, 1942

Order Enoplida Chitwood & Chitwood, 1937

Suborder Enoplina Chitwood & Chitwood, 1937

Superfamily Enoploidea Dujardin, 1845

Family Thoracostomopsidae Filipjev, 1927

Subfamily Enoplolaiminae De Coninck, 1965

Genus *Epacanthion* (Wieser, 1953)

Genus *Mesacanthion* Filipjev, 1927

Diagnosis of Thoracostomopsidae Filipjev, 1927 (after Smol & Coomans, 2006)

Lips high. Only dorsolateral orthometanemes with a robust scapulus but no caudal filament. Inner labial sensilla robust and setiform (papilliform only in *Fenestrolaimus*), outer labial and cephalic setae robust and long. Epidermal glands well-differentiated outlet. Inner layer of cuticle forms cephalic capsule on to which pharyngeal muscles are attached. Inner layer of cuticule forms a cephalic capsule onto which pharyngeal muscles are attached. Cephalic organs often present, and of variable shape. Amphids small and situated posterior to the

cephalic capsule, or absent. Spacious buccal cavity with three mandibles and three teeth (one dorsal and two ventro-sublateral) or with one long eversible spear. Female reproductive system didelphic–amphidelphic with antidromously reflexed ovaries (a single posterior ovary in *Mesacanthion monhystera* only). Caudal glands penetrate into the precaudal region.

Diagnosis of the genus *Epacanthion* Wieser, 1953 (after Smol & Coomans, 2006)

Enoplolaiminae. Cuticle usually smooth. Head broadly wedge- or cone-shaped. Lips high, mostly striated. Inner labial setae long and inserted at the base of lip flaps; outer labial and cephalic setae situated at middle or anterior end of cephalic capsule. Cervical setae often present and can be numerous in males, which exhibit sexual dimorphism. Mandibles consisting of two plate-shaped columns (usually long and parallel) separated by a thin sheet of cuticle (space between columns not solid) and only connected anteriorly by a bar (an intermediate stage between *Enoploides* and *Mesacanthion*); mandibular teeth small, with gland opening at tip. Pharynx relatively long and cylindrical; cardia pyriform. Females didelphic-amphidelphic with reflexed ovaries at left side of the intestine. Males diorchic with both testes at left side of intestine. Spicules mostly long (≥ 2.5 anal diameters) or short; gubernaculum with apophyses present or absent. Pre-anal supplement present or absent. Three caudal glands, cells pre-caudally. Tail narrowly conical or attenuated. Marine.

List of valid (according to Guilherme *et al.*, 2009)

- *Epacanthion agubernaculus* Guilherme *et al.*, 2009
- *Epacanthion brevispiculosum* Mawson, 1958
- *Epacanthion brevispiculum* Mawson, 1956
- *Epacanthion bütschlii* (Southern, 1914)
- *Epacanthion durapelle* (Kreis, 1929)
- *Epacanthion enoploidiforme* (Gerlach, 1953)
- *Epacanthion exploratoris* Greenslade & Nicholas, 1991
- *Epacanthion flagellicaudum* Gerlach, 1956
- *Epacanthion galeatum* Boucher, 1977
- *Epacanthion georgei* Inglis, 1971
- *Epacanthion gorgonocephalum* Warwick, 1970
- *Epacanthion mawsoni* Warwick, 1977
- *Epacanthion microdentatum* Wieser, 1953
- *Epacanthion multipapillatum* (Wieser, 1959)
- *Epacanthion murmanicum* (Ssaweljev, 1912)
- *Epacanthion nadjae* Sergeeva, 1974
- *Epacanthion oliffi* Inglis, 1966
- *Epacanthion oweni* Keppner, 1986

- *Epacanthion pellucidum* (Ssawejev, 1912)
- *Epacanthion polysetosum* (Jensen, 1986)
- *Epacanthion saveljevi* (Filipjev, 1927)
- *Epacanthion stekhoveni* Greenslade & Nicholas, 1991

Epacanthion canceriformis sp. nov. (Table 1, Figures 4 and 7)

Studied material: 16 males and 6 females.

Type material: Male holotype, found in Potiguar Basin (Rio Grande do Norte, Brazil), at 5.9 meters depth, in sand with medium grain size and calcareous and organic fragments, collected with a box corer. National Museum of Rio de Janeiro, Brazil (MNRJ 342).

Allotype female: found in Potiguar Basin (Rio Grande do Norte, Brazil) at 6.3 meters depth, in coarse white sand, collected with a box-corer. National Museum of Rio de Janeiro, Brazil (MNRJ 343).

Paratypes: 15 males and 5 females found in Potiguar Basin (Rio Grande do Norte, Brazil), at 5.9-40.7 meters depth, in sand with medium grain size and calcareous and organic fragments, collected with a box corer. Meiofauna Laboratory of the Zoology Department, Universidade Federal de Pernambuco (157-176 NM LMZOO-UFPE).

Measurements: See Table 1.

Etymology: Species name (*canceriformes*) based on the main morphological body feature: the shape of the gubernaculum resembling a crab's pincer.

Description (Figures 3, 4, 5, 6 and 7)

Long body, narrowing at the extremities (L= 5319.6 μm long), cuticle finely striated. Cephalic capsule (64 μm wide) well sclerotized. Cephalic arrangement in three separate circles, 6 inner labial setae (21 μm), 6 outer labial setae longer (37.2 μm) than 4 cephalic setae (23.4 μm), and both inserted in middle of cephalic capsule. Subcephalic setae present (24 μm). Cervical setae concentrated after level of nerve ring (4.2-19.8 μm). Somatic setae present (9-37.5 μm). Buccal cavity with high lips and semilunar striation. Three solid mandibles (27 μm long and 4.8 μm wide), well sclerotized, three solid teeth (12 μm) of the same size. Pore of cervical glands in base of each tooth. Secretory-excretory pore at 64.8 μm from posterior end. Amphids pocket-shaped (12.6 μm wide), 18.2 % of corresponding body diameter, 55.5 % from anterior end. Cephalic organ present. Pharynx strongly muscular (1153.2 μm long),

21.6% from anterior end. Nerve ring (72 μm) in anterior portion of pharynx, 18.8% from anterior end, occupying 77.7% of total pharynx length. Intestine communicating with pharynx through cardia. Cardia pear-shaped. Two testes to left of intestine. Spicules (52.5 μm long) bottle-shaped, proximal and distal regions narrow with little cephalization on tip, expanded in middle. Muscle bundles connected to distal portion of spicule. Gubernaculum short (12.9 μm), sclerotized, pincer-shaped, connected to spicules, without apophysis. Single tubular pre-cloacal supplement (12 μm), at 452.6 μm from cloaca. Conical-cylindrical tail (272.8 μm long) with several setae (48 - 11.4 μm) along its length. Tubular spinneret. Two pairs of setae on tail tip (15 μm). Three caudal glands, one of them at same level as cloaca.

L= 5319.6 μm; a = 59.1 μm; b = 4.6 μm; c = 19.5 μm; c' = 4.7 μm.

Allotype: Female similar to male. Body length (4914 μm). Head width (63 μm). Inner labial setae (25.8 μm), outer labial setae (46.8 μm), and cephalic setae (23.4 μm). Somatic setae (24 μm-7.2 μm). Mandibles (29.4 μm long and 7.2 μm wide). Pharynx (1302 μm), nerve ring (74.2 μm). Reproductive system didelphic, with ovaries opposed and reflexed. Tail conical-cylindrical (310 μm).

L= 4914 μm; a = 42 μm; b = 3.7 μm; c = 15.8 μm; c' = 5.1 μm.

Table 1. Measurements of males of *Epacanthion canceriformis* sp. nov. All measurements in μm and in the form: mean (range).

Table 1. Mesures chez les mâles de *Epacanthion canceriformis* sp. nov. Toutes les mesures en μm et sur la forme: moyenne (variation).

Parameter	Male		Female	
	Holotype	Paratypes	Allotype	Paratypes
n	1	15	1	5
L	5319.2	4912(3900-5722.20)	4914	4192(3588-4492.8)
hd	60	68 (51- 87)	63	59.3(52.5-69.1)
amph	12.6	14.4 (11.4-16.8)	12	12.6 (12-13.2)
amph %	18.2	23 (13.4-29.4)	18	18 (16.5-19.4)
amph. pos	56	52 (28-71)	53	53.3 (45.6-59.4)
mabw	27	31.3 (27-39)	29.4	29 (25.8-32.4)
mabl	4.8	6 (4.8-6.8)	7.2	5.6 (3.2-7.2)
nr	72	83 (63-111)	74.2	66 (55.5-74.2)
ph	1153.2	1228.3 (1048-1277.2)	1302	1115 (1091.2-1302)
mbd	90	101 (79.5-117)	117	98 (72-118.5)
spic	52.5	61 (51.3-70.5)	-	-
v	-	-	2808	2250.1 (1732.8-808.0)
V%	-	-	57.1	56.2
supl	12	14.4 (12-16.2)	-	-
abd	57	72 (57-130)	60	60 (45-67.5)
c.s.	23.4	31.2 (11.4-57.6)	23.4	22.3 (19.8–26.4)
gub.	10.5	13.6 (9.4-18)	-	-
t	272.8	268 (235.6-291.4)	310	289 (272.8–312)
som s	37.5	21 (12-19.5)	21	20 (9-21)
cerv.s	19.8	19 (12.9 – 28)	23.4	13.8 (6-23.4)
s'cefs	23.4	29 (13.8 – 49.5)	-	-
a	59.1	49 (36.7 – 60.2)	42	43.6 (37.9–55.4)
b	4.6	4.0 (3.3 – 4.6)	3.7	3.7 (3.0–4.1)
c	19.4	18.3 (14.9 – 20.8)	15.8	14.5 (12.4–16.1)
c'	4.7	4.0 (3.3-4.7)	5.1	5.0 (4.0-5.1)

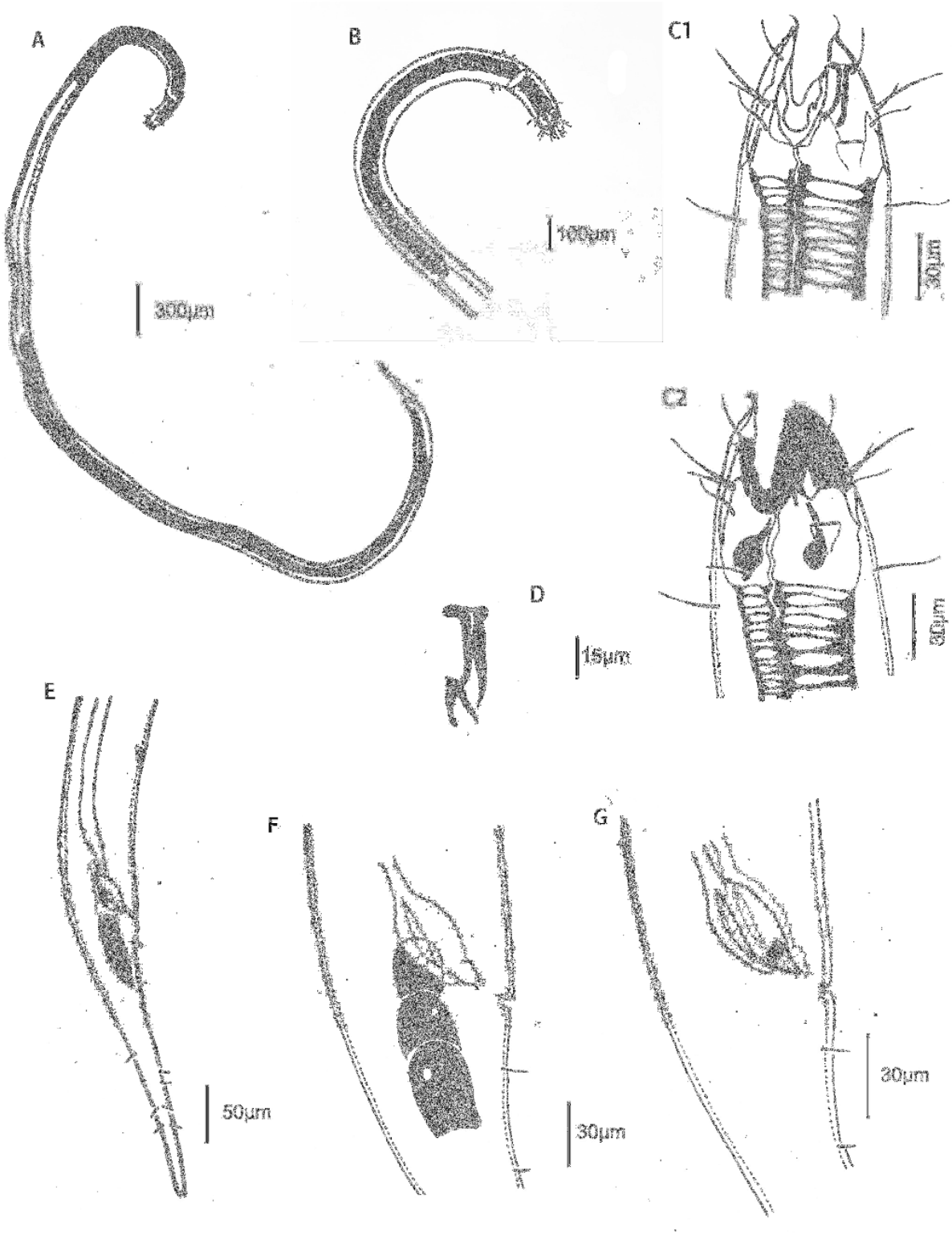

Figure 3. Male. *Epacanthion canceriformis* sp. nov. A. Total body, B. Anterior region, C1. Head, amphid and the buccal cavity with mandibles, C2. Head with semilunar striation and teeth, D. Mandible detail, E. Setae in the tail region, gland and supplement, F. Spicule and glands, G. Spicule.

Figure 3. Mâle. *Epacanthion canceriformis* sp. nov. A. Aspect général du corps, B. Région antérieure avec glandes oesophagiques, C1 Tête, amphide et cavité bucale avec mandibules. C2. Tête avec stries semilunaires et

dent, D. Détail de la mandibule, E. Soie en la région de la queue, glande et supplément F. Spicules et glandes G. Spicule.

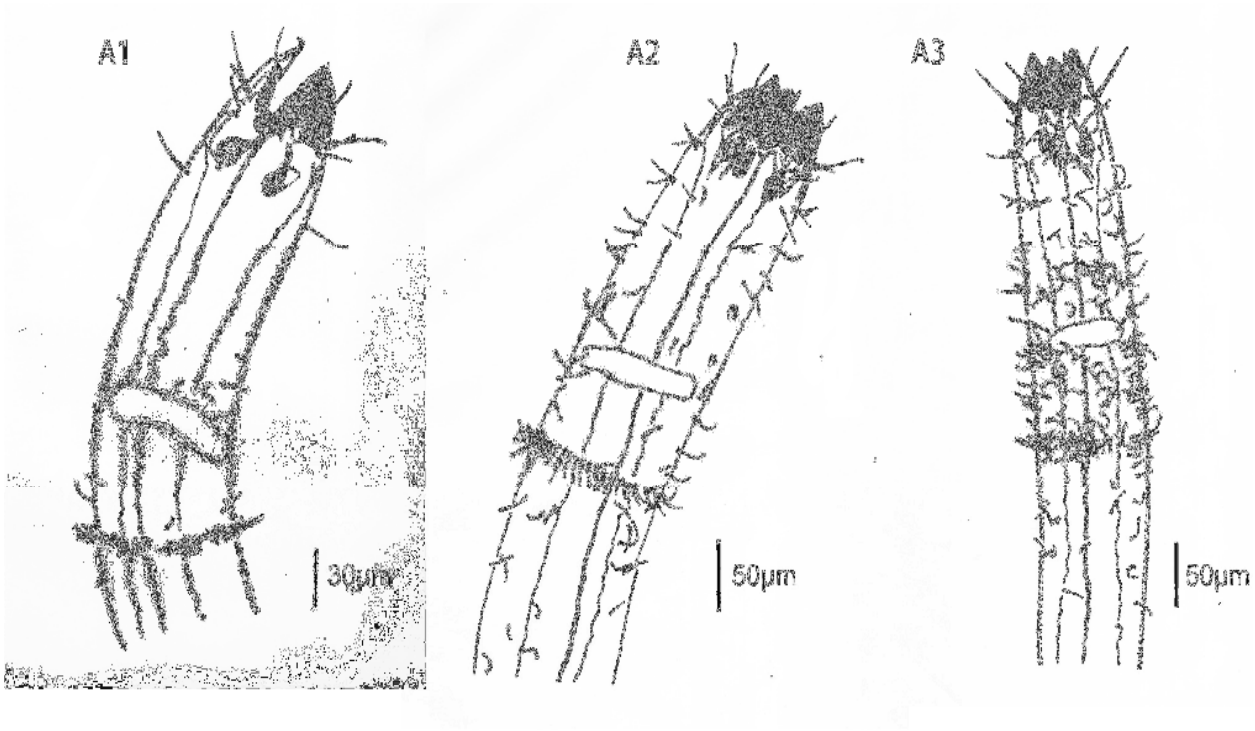

Figure 4. Male. *Epacanthion canceriformis* sp. nov. A1, A2. and A3. Anterior region with detail of cervical setae

Figure 4. Mâle. *Epacanthion canceriformis* sp. nov A1, A2 and A3. .Région antérieure avec détail et soies cervicales

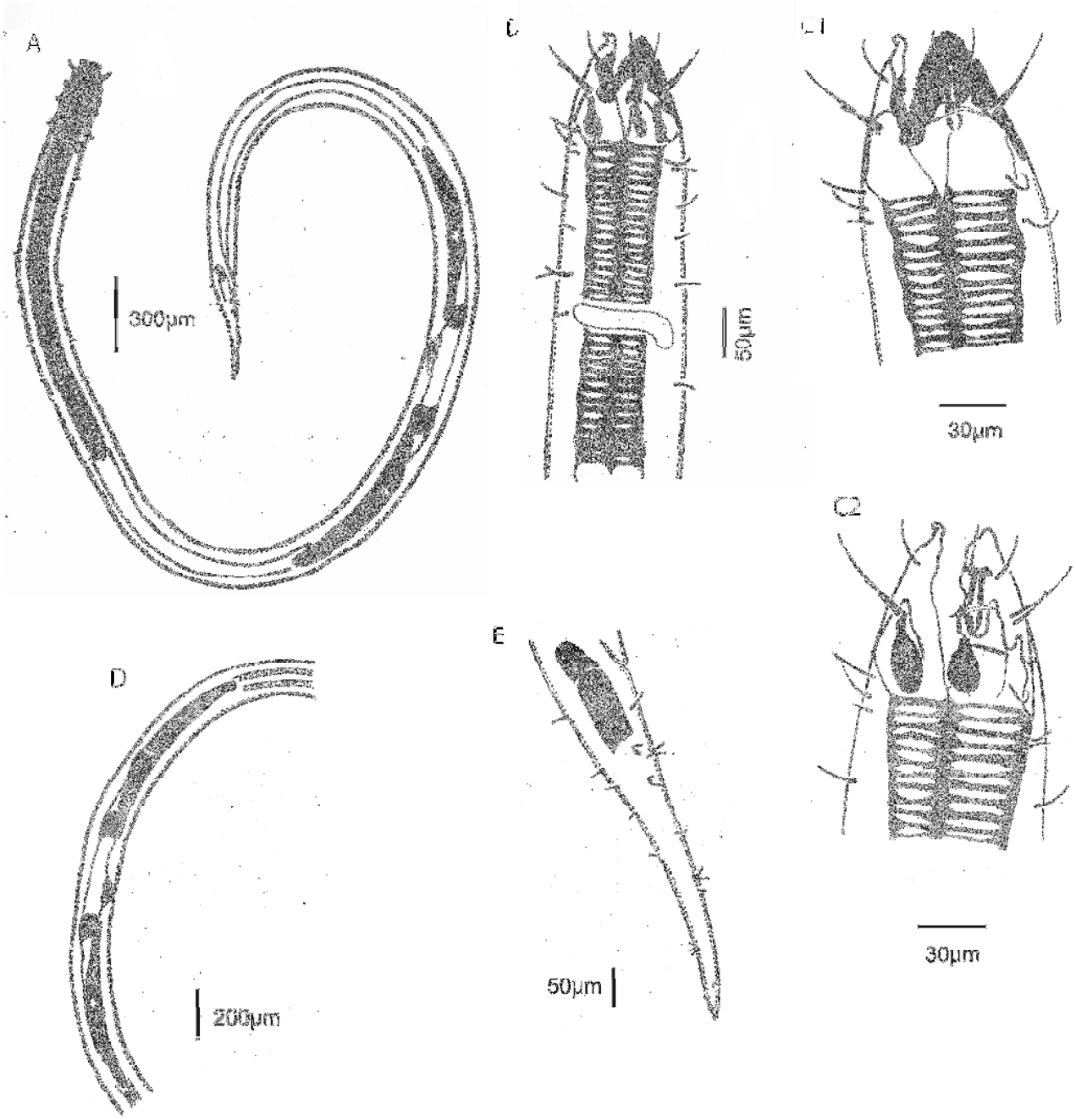

Figure 5. Female. *Epacanthion canceriformis* sp. nov. A. Total body, B. Anterior region, C1. Head with semilunar striation and teeth, C2. Head, amphid and the buccal cavity with mandibles, D. vulva region, E. tail region.

Figure 5. Femelle. *Epacanthion canceriformis* sp. nov A. Aspect général du corps, B. Région antérieure, C1. Tête avec stries semilunaires et dent, C2. Tête, amphide et cavité bucale avec mandibules, D. Région de la vulve, E. Queue.

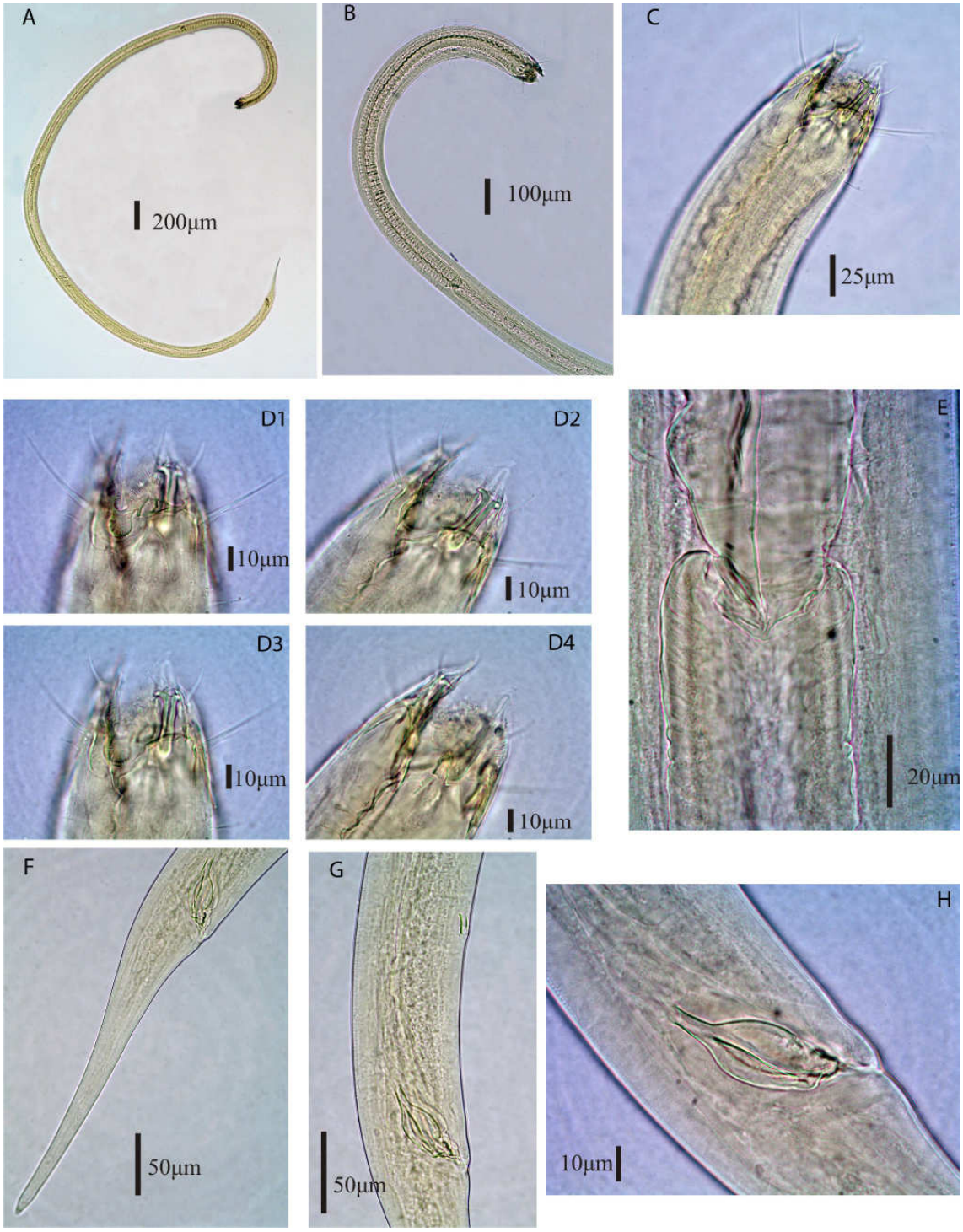

Figure 6. Male. *Epacanthion canceriformis* sp. nov. A. Total body, B. Anterior region, C. Head and the buccal cavity with mandibles, D1. Mandible detail, D2. mandibles and Teeth, D3 and D4 Head with semilunar striation and teeth, E. Detail of cardia, F. Tail region, G. Spicules and supplement. H. Detail of spicules.

Figure 6. Mâle. *Epacanthion canceriformis* sp. nov. A. Aspect général du corps, B. Région antérieure, C. Tête, amphide et cavité bucale avec mandibules, D1. Détail de la mandibule, D2. Détail de la mandibule et dent, D3 et D4. Tête avec stries semilunaires et dent, E. Détail de cardia, F. Queue, G. Spicule et supplement, H. détail de la spicule.

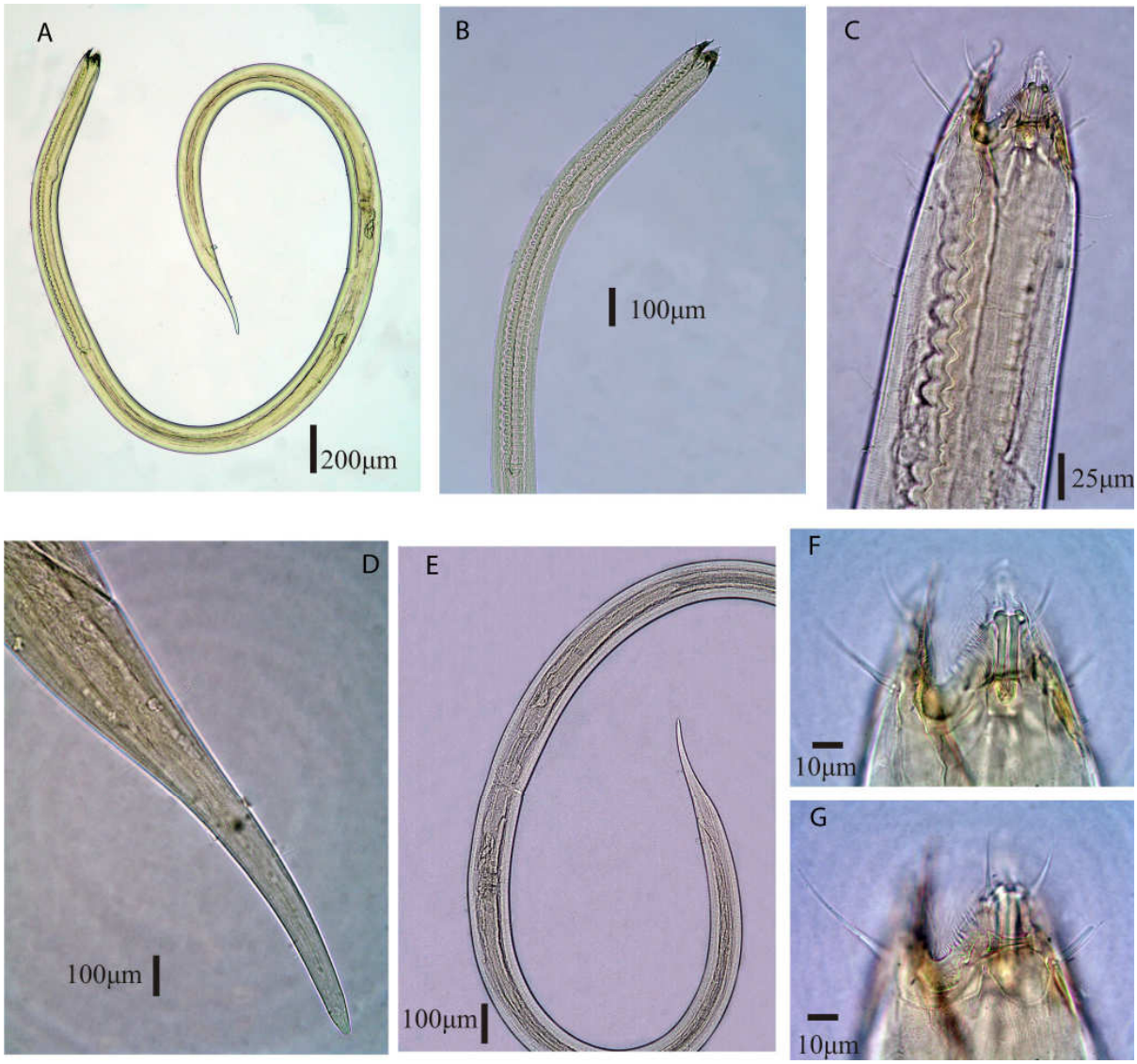

Figure 7. Female. *Epacanthion canceriformis* sp. nov. A. Total body, B. Anterior region, C. Head detail, D. tail region, E. vulva region, F. Head, detail of the buccal cavity with mandibles, G. Head with semilunar striation and teeth.

Figure 7. Femelle. *Epacanthion canceriformis* sp. nov. A. Aspect général du corps, B. Région antérieure, C. détail de la tête, D. Détail de la queue, F. Tête, détail et cavité bucale avec mandibules, G. Tête avec stries semilunaires et dent.

Discussion

The new species shares the same diagnostic features for the genus *Epacanthion* with all the valid species of this group: cephalic capsule strongly sclerotized, setae inserted in the middle of the cephalic capsule, three teeth and three mandibles heavily sclerotized, with two solid longitudinal bars jointed by a thin sheet (Smol & Coomans, 2006).

The mandibles and oesophageal glands that open through each tooth, are similar to the features of *Epacanthion bütschlii, E. durapelle,* and *Epacanthion agubernaculus*. The most important difference between the new species and the previously described species is in the shape of the spicules. The differences in the shape and length of the spicules in *Epacanthion* were studied by Wieser (1959) and Greenslade & Nicholas (1991). Wieser (1959) considered that the length of spicules was one of the most important attributes, and classified the species according to the size of the copulatory organ, splitting the species into two groups: those that possess longer spicules, and the others with shorter spicules. Greenslade & Nicholas (1991) constructed a key based on their own original and other published descriptions. The authors compiled several and specific features considered important and usefulness for the genus. Among these, the first character listed was the length and shape of the spicules.

All the specimens showed variation in the distribution of the somatic setae, which are numerous in the cervical region and have different lengths. After the nerve ring they assume a well-defined transverse arrangement. Posterior to this region, the setae are sparser, then increase in number again in the caudal region. The females have fewer setae than the males. This new species resembles *E. butschlii, E. durapelle, E. galeatum, E. georgei, E gorgonocephalum, E. oliffi,* and *E. oweni* in having a different arrangement of the cervical setae along the pharynx. The number and distribution of the cervical setae are reduced in the females, and this feature is also observed in the females of *E. gorgonocephalum* and *E. oliffi*.

Epacanthion canceriformis sp. nov. has the same arrangement (6+6+4) and position of the setae on the cephalic capsule as the majority of the species in this genus, but in relation to both features it differs from *E. agurbenaculus, E. bütschlii, E. enoploidiforme, E. gorgonocephalum, E. multipapillatum, E. pellucidum,* and *E. polysetosum*.

Besides the length of the spicules, the shape is also different in the new species; it is ‘bottle-shaped’ with striations in the distal portion. The gubernaculum is connected to the spicules, a feature not previously observed in any other species of *Epacanthion*. Within the family Thoracostomopsidae, this character is found in *Enoplolaimus connexus* Wieser, 1953a and *Enoploides longispiculosus* Vitiello, P. 1967.

In the new species, the caudal setae extend along the entire length. The presence of the setae along the tail is also observed in almost all valid species of *Epacanthion*, except in *E. flagellicaudum* and *E. polysetosum*. However, the description of the new species is based on several specimens (16 males and 6 females) that show extensive variation; and this could be considered sufficient to establish these individuals as a valid taxon.

Diagnosis of the genus *Mesacanthion* Filipjev, 1927 (after Smol & Coomans, 2006)

Enoplolaiminae. Outer labial and cephalic setae situated at middle or anterior end of cephalic capsule. Mandibles well developed, provided with claws, arch-shaped, consisting of two rod-like columns anteriorly united by a curved bar. Teeth shorter than mandibles. Spicules mostly short, if long (*M. diplechma*) then gubernaculum with caudal apophysis. One freshwater species and one brackish-water species: *M. longispiculum* Gerlach, 1954 [described as *M. cf. longispiculum* and found in oligohaline lagoon in Madagascar by Gerlach (1958)].

According to the NeMys database (Deprez *et al*., 2005)**,** there are 37 valid species of *Mesacanthion*:

- *Mesacanthion africanthiforme* Warwick, 1970
- *Mesacanthion africanum* Gerlach, 1957
- *Mesacanthion agubernatus* Vitiello, 1971
- *Mesacanthion arcuatile* Timm, 1961
- *Mesacanthion armatum* Wieser, 1959
- *Mesacanthion audax* Stekhoven, 1935
- *Mesacanthion banale* Filipjev, 1927
- *Mesacanthion brachycolle* Allgén 1959
- *Mesacanthion breviseta* Filipjev, 1927
- *Mesacanthion cavei* Inglis, 1964
- *Mesacanthion ceeum* Inglis, 1964
- *Mesacanthion conicum* Filipjev, 1918
- *Mesacanthion cricetoides* Wieser, 1959
- *Mesacanthion diplechma* Boucher, 1977
- *Mesacanthion fricum* Inglis, 1966
- *Mesacanthion gracilisetosus* Alggén, 1930
- *Mesacanthion hawaiiensis* Alggén, 1951
- *Mesacanthion hirsutum* Gerlach, 1953
- *Mesacanthion infantile* Ditlevsen 1930
- *Mesacanthion karense* (Filipjev, 1927)
- *Mesacanthion kerguelense* Mawson, 1958
- *Mesacanthion longispiculum* Gerlach, 1958
- *Mesacanthion longissimesetosum* Wieser, 1953
- *Mesacanthion lucifer* Filipjev, 1927
- *Mesacanthion majus* Wieser, 1953
- *Mesacanthion monhystera* Gerlach, 1967

- *Mesacanthion Obscurum* Gargarin & Klerman, 2006
- *Mesacanthion pacificum* Alggén, 1951
- *Mesacanthion pali* Wieser, 1959
- *Mesacanthion pannosum* Wieser, 1953
- *Mesacanthion paradentatus* Alggén, 1932
- *Mesacanthion proximum* Gerlach, 1957
- *Mesacanthion rigens* Gerlach, 1957
- *Mesacanthion southerni* Warwick, 1973
- *Mesacanthion tenuicaudatum* Ssaweljev, 1912
-*Mesacanthion ungulatum* Wieser, 1953
-*Mesacanthion virile* Diltevsen, 1930

***Mesacanthion henriquei* sp. nov.** (Table 2, Figures 8 and 11)

Studied material: 15 males and 6 females.

Type material: Male holotype, found in Potiguar Basin (Rio Grande do Norte, Brazil). No information about depth and sediment. Collected with box corer, and Van Veen. National Museum of Rio de Janeiro, Brazil (MNRJ 344).

Allotype female: found in Potiguar Basin (Rio Grande do Norte, Brazil). No information about depth and sediment. Collected with box corer and Van Veen. Same collection data as holotype. National Museum of Rio de Janeiro, Brazil (MNRJ 345).

Paratypes: 14 males and 5 females, found in Potiguar Basin (Rio Grande do Norte, Brazil). No information about depth and sediment. Collected with box corer and Van Veen. Meiofauna Laboratory of the Zoology Department, Universidade Federal de Pernambuco (177-193 NM LMZOO-UFPE).

Measurements: See Table 2.

Etymology: The species name honors Pedro Henrique Guilherme da Costa Rego, son of the first author.

Description (Figures 8, 9, and 10)

Long body, narrowing at the extremities (L= 4820.4 µm long). Cuticle finely striated. Cephalic capsule well-defined and strongly sclerotized. Cephalic arrangement in three separate circles: 6 inner labial setae (17.4 µm), 6 outer labial setae longer (42.6 µm) than 4 cephalic setae (22.2 µm), positioned slightly above to middle of cephalic capsule. Cephalic organ rounded. Low lips and three arc-shaped sclerotized mandibles (33.6 µm long and 14.4 µm wide) with two lateral sclerotized rods united by curved bar inside buccal cavity. Three

well-defined onchia, with same length. Cervical glands on base of cephalic capsule, opening in each tooth. Ventral gland close to nerve ring, secretory-excretory pore at 58.5 μm from anterior end. Amphids pocket-shaped (10.5 wide), 42 μm from anterior, 13.3% of corresponding body diameter. Pharynx strongly muscular (899 μm). Nerve ring with 66 μm at 26.5% from anterior end, occupies 68.7% of total pharynx length. Long (483.6 μm) and striated spicules with wall strongly sclerotized. Two testes in tandem, the larger measuring 1006.5 μm and the smaller 345 μm. Gubernaculum sclerotized (43.5 μm), without apophysis. Only one cylindrical precloacal supplement (65 μm). Postcloacal papillae with setae and pore joined to glands. Conical-cylindrical tail (186 μm) with five setae (31.2 μm). Tubular spinneret (3 μm). Three caudal glands observed.

L= 4820.4 μm; a = 40.6 μm; b = 5.3 μm; c = 25.9 μm; $c^{'}$ = 3.1 μm.

Allotype female: Similar to male. Total body length (4056 μm). Head width (69 μm). Inner labial setae (19.2 μm), outer labial setae (43.8 μm), cephalic setae (24 μm). Mandibles (38.4 μm long and 17.4 μm wide). Pharynx (1128.4 μm) with nerve ring (75 μm). Secretory-excretory pore not observed. Reproductive system didelphic, with ovaries opposed and reflexed.

L= 4914 μm; a = 33.4 μm; b = 3.5 μm; c = 15.9 μm; $c^{'}$ = 4.2 μm.

Table 2. Measurements of females of *Mesacanthion henriques* sp. nov. All measurements in μm and in the form: mean (range).

Table 2. Mesures chez les femelles de *Mesacanthion henriques* sp. nov. Toutes les mesures en μm et sur la forme: moyenne (variation).

Parameter	Male		Female	
	Holotype	Paratypes	Allotype	Paratypes
n	1	12	1	5
L	4820.4	3917 (2652-5116.8)	4056	3942 (3135.6-4492.8)
hd	52.5	52.4 (37.5-96)	69	64 (45-82.5)
amph	10.5	11.4 (9-16.2)	18	15.2 (12-22.8)
amph %	13.3	22.4 (22.7-26.9)	24	22 (16-30.4)
amph. pos	42	37 (27-54.6)	46.8	48 (38.4-67.5)
mabw	33.6	31(23.46.2)	38.4	35.3 (24.6-43.8)
mabl	14.4	13(9-16.8)	17.4	24 (19.8-24)
nr	66	63 (45-84)	75	71 (54-81)
ph	899	747 (496-1104)	1128.4	826 (595.2-1128.4)
mbd	118.5	103.4 (75-120)	121.5	129 (115.5-142.5)
spic	483.6	670.3 (483.6-954.8)	-	-
v	-	-	2823.6	2194.0 (1310.4-2823.6)
V%	-	-	69.7	55.4 (41.7-69.7)
supl	65	69.5 (37.5-85.5)	-	-
abd	63	47.3 (37.5-63)	60	54 (34.5-66)
c.s.	22.2	22 (13.2-48)	24	21.1 (16.8-24)
gub.	43.5	49 (40.5-81)	-	-
t	186	212.2 (167.4-390)	254.2	251.1
a	40.6	38 (24.7-56)	33.4	31 (25.2-33.4)
b	5.3	5.2 (4.6-5.7)	3.5	4.8 (3.5-5.8)
c	25.9	19.3 (7.6-25.9)	15.9	1.6 (12.5-19.6)
c’	3.1	4.6 (3.1-10.4)	4.2	4.7 (4.2-5.9)

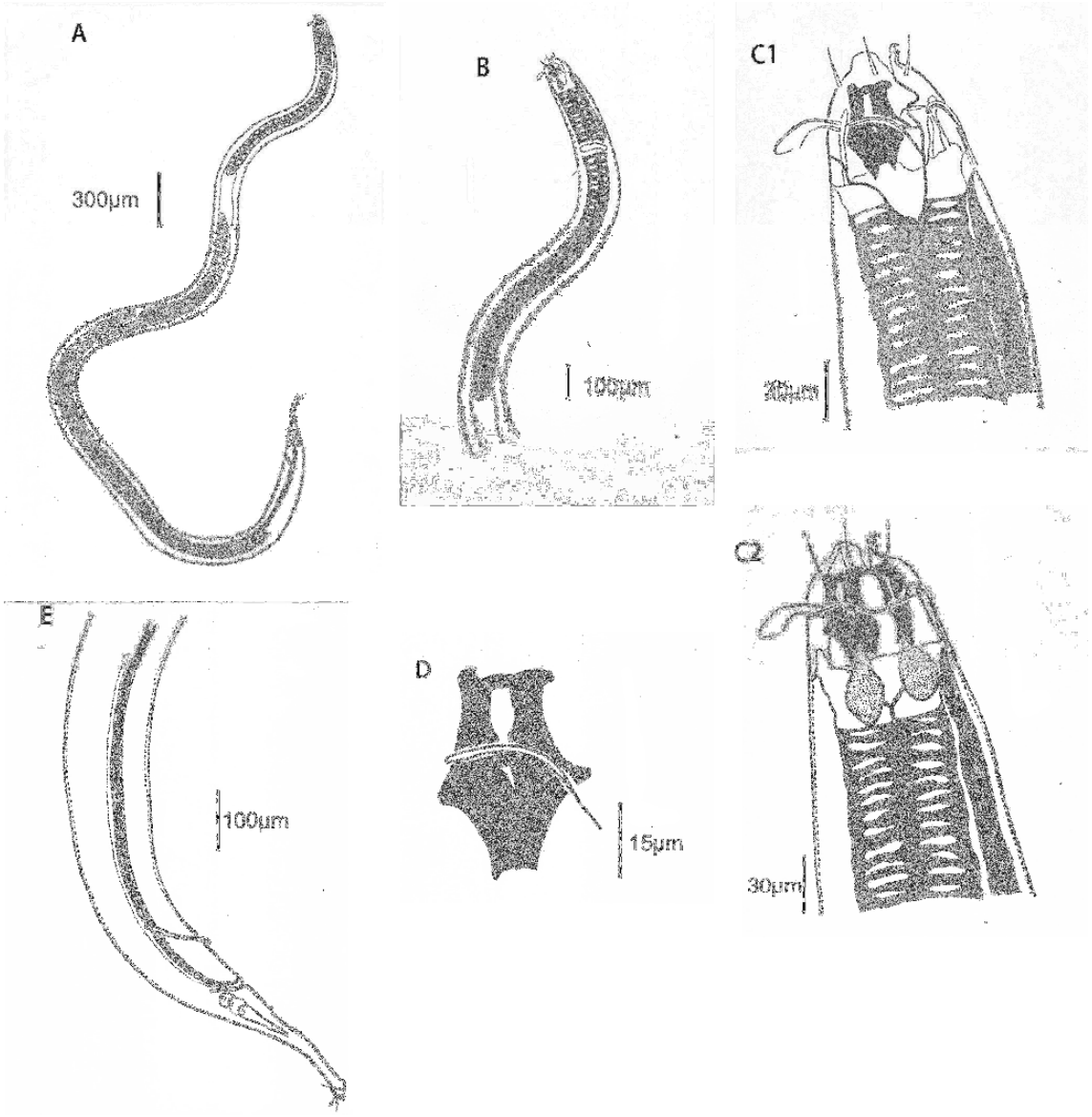

Figure 8. Male. *Mesacanthion henriquei* sp. nov. A. Total body, B. Anterior region with the esophageal glands, C1. Head detail, C2. Head and the buccal cavity with mandibles and glands, D. Mandible detail, E. Spicules, tail, and supplement.

Figure 8. Mâle. *Mesacanthion henriquei* sp. nov. A. Aspect général du corps, B. Région antérieure et glande, C1. Détail de la tête, C2. Tête avec cavité bucale avec mandibules et glande, D. Détail de la mandibule, E. Spicules, queue et supplément.

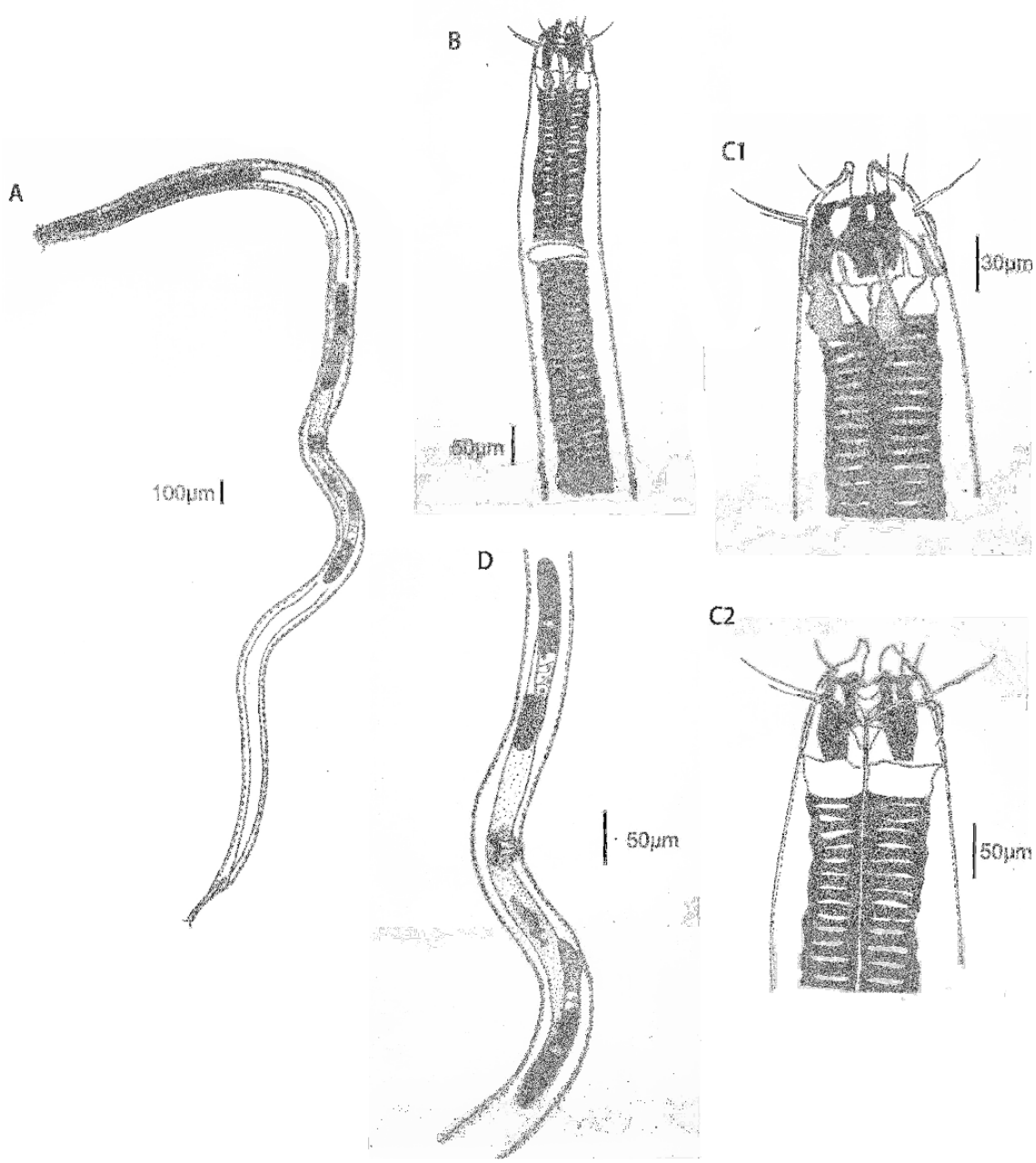

Figure 9. Female *Mesacanthion henriquei* sp. nov. A. Total body, B. Anterior region, C1. Head and the buccal cavity with mandibles and glands, C2. Head detail, D. Détail of the vulva region.

Figure 9. Femmele. *Mesacanthion henriquei* sp. nov.A. Aspect général du corps, B. Région antérieure, C1. Tête avec cavité bucale avec mandibules et glande, C2. Détail de la tête., D. Détail et région de la vulve.

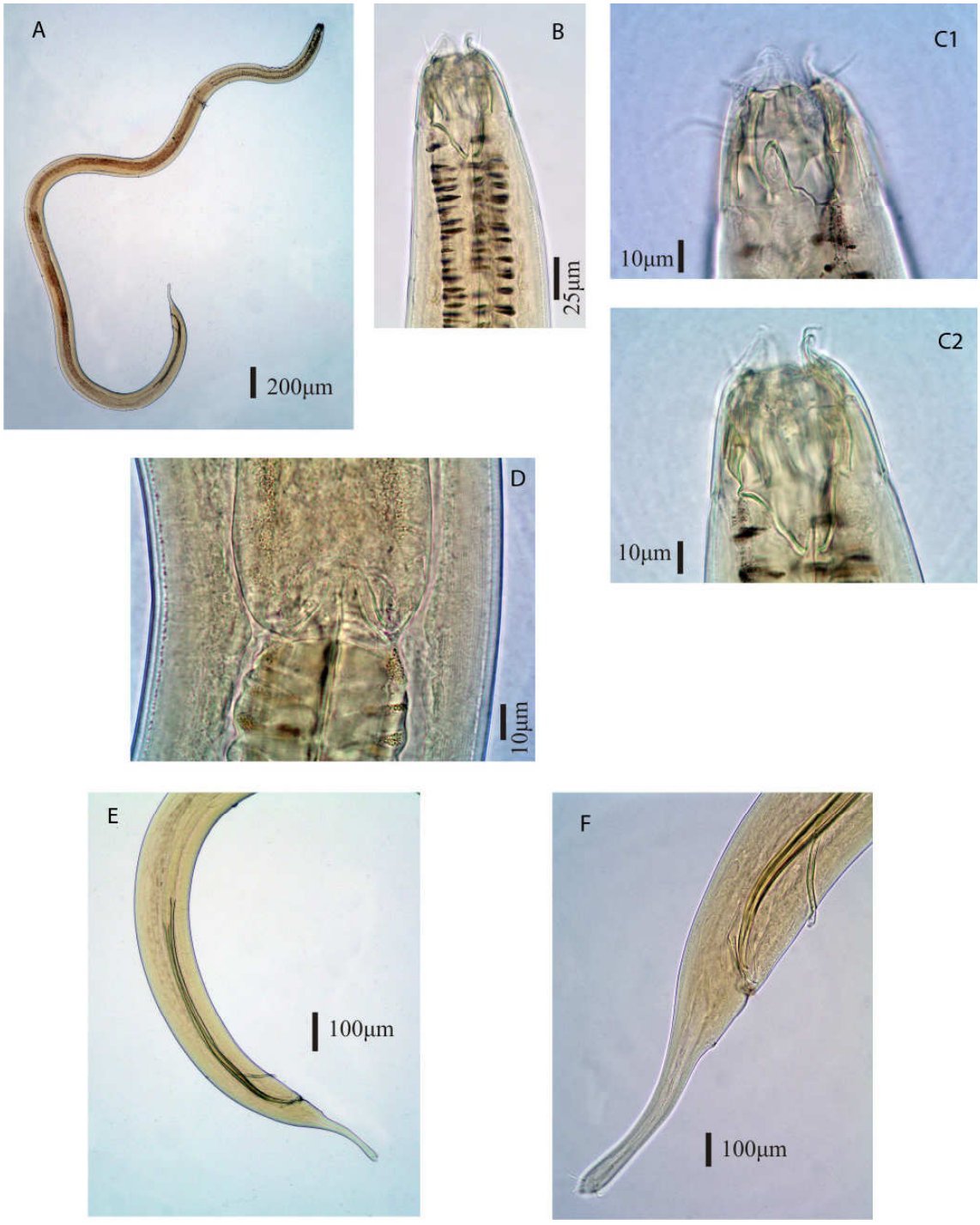

Figure 10. Male *Mesacanthion henriquei* sp. nov. A. Total body, B. Anterior region, C1. And C2. Head and the buccal cavity with mandibles and glands, D. Detail of cardia, E. Detail of spicules, F. Spicules, tail, and supplements.

Figure 10. Mâle. *Mesacanthion henriquei* sp. nov. A. Aspect général du corps, B. Région antérieure, C1. et C2. Tête avec cavité bucale avec mandibules et glande, D. Détail de la cardia, E. Détail et spicules, F. Spicules, queue et supplément.

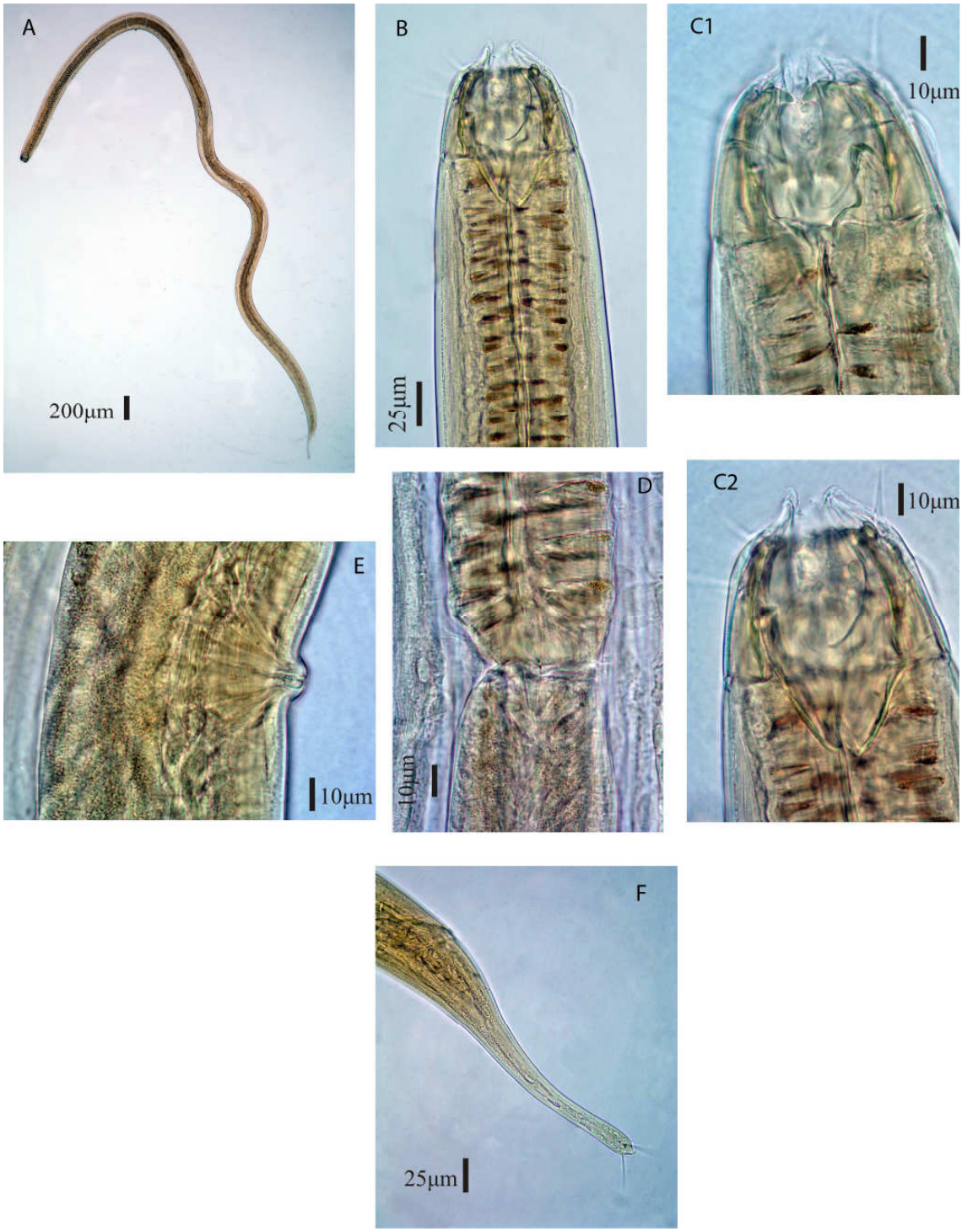

Figure 11. Female. *Mesacanthion henriques* sp. nov. A. Total body, B. Anterior region C1 and C2. Head and the buccal cavity with mandibles and teeth, D. Detail of cardia, E. Detail of vulva, F. Tail region and tail setae.

Figure 11 Femelle *Mesacanthion henriquei* sp. nov.A. Aspect général du corps, B. Région antérieure, C1. et C2. Tête avec cavité bucale avec mandibules et dent, D. Détail de la cardia, E. Détail et vulve, F. Région de la queue.

***Mesacanthion dentantuspiculum* sp. nov.** (Table 3, Figure 11)

Studied material: 4 males.

Type material: Male holotype, found in Potiguar Basin (Rio Grande do Norte, Brazil). No information about depth and sediment. Collected with box corer and Van Veen. National Museum of Rio de Janeiro, Brazil (MNRJ 346).

Measurements: See Table 3.

Etymology: Specific name (*dentantuspiculum*) based on the main morphological body feature: denticles on the distal portion of the spicules.

Description (Figure 10)

Long body, narrowing at extremities (L= 4056 μm). Cuticle finely striated. Cephalic capsule well sclerotized (54 μm). Cephalic arrangement with three separated circles (6+6+4), six inner labial (23.4 μm), six outer labial longer (75 μm) than four cephalic setae (38.4 μm) positioned in middle of cephalic capsule. Lips low, with equal mandibles (27 μm long and 13.2 μm wide), joined by solid longitudinal bar. Mandibles with three teeth of same length (16.8 μm), well defined. Cervical glands on base of cephalic capsule, opening in each tooth. Ventral gland below nerve ring at 25.2 μm from anterior end. Pharynx strongly muscular and sclerotized (1023 μm). Somatic setae present along pharynx. Nerve ring (78 μm) positioned anteriorly, occupying 80% of corresponding body diameter. Triangular cardia. Amphids pocket-shaped (13.2 μm wide), at 23.4 μm from anterior end, 22.8% of corresponding body diameter. Two opposed testes, the larger 493 μm) right of the intestine and the smaller left of the intestine (439.5 μm). Spicules short (136.5 μm), strongly sclerotized, muscle fibers connected to proximal portion of spicule and on apophysis of gubernaculum. Anterior region of spicules open, denticles observed on proximal, distal, ventral, and dorsal regions. Bifid distal region. Gubernaculum strongly sclerotized (111 μm), muscle fibers connected to body wall. Dorso-caudal apophysis, occupies 79.7% of gubernaculum length, distal region appears warty on tip. Single tubular supplement (28.5 μm), 178.5 μm from cloaca. Conical tail (347.2 μm). Three glands before the cloaca. Tubular spinneret.

L= 4056 μm; a = 29 μm; b = 4.1 μm; c =11.6 μm; c' = 3.7 μm.

Females and juveniles not found.

Table 3. Measurements of males of *Mesacanthion dentantuspiculum* sp. nov. All measurements in μm and in the form: mean (range).

Table 3. Mesures chez les mâles de *Mesacanthion dentantuspiculum* sp. nov. Toutes les mesures en μm et sur la forme: moyenne (variation).

Parameter	Male	
	Holotype	Paratypes
n	1	3
L	4056	4450 (4212-5054.4)
hd	54	61 (60-67.5)
amph	13.2	20 (13.2-28.5)

amph %	22.8	32.1 (20.5-47.5)
amph. pos	23.4	32 (23.4-39)
mabw	27	28.2 (27-30)
mabl	13.2	14 (12.6-15)
nr	78	84 (78-93)
ph	1023	1077.3 (1023-1140.8)
mbd	139.5	139.1 (135-139.5)
spic	136.5	147.3 (136.5-153)
v	-	-
V%	-	-
supl	28.5	29.2 (22.5-36)
abd	93	94.5 (93-99)
c.s.	38.4	43 (38.4-48.6)
gub.	111	107 (102-108)
t	347.2	391(347.2-409.2)
a	29	32 (29-37)
b	4.1	4.1 (3.6-4.7)
c	11.6	11.3(10.2-12.5)
c’	3.7	4.1 (3.7-4.4)

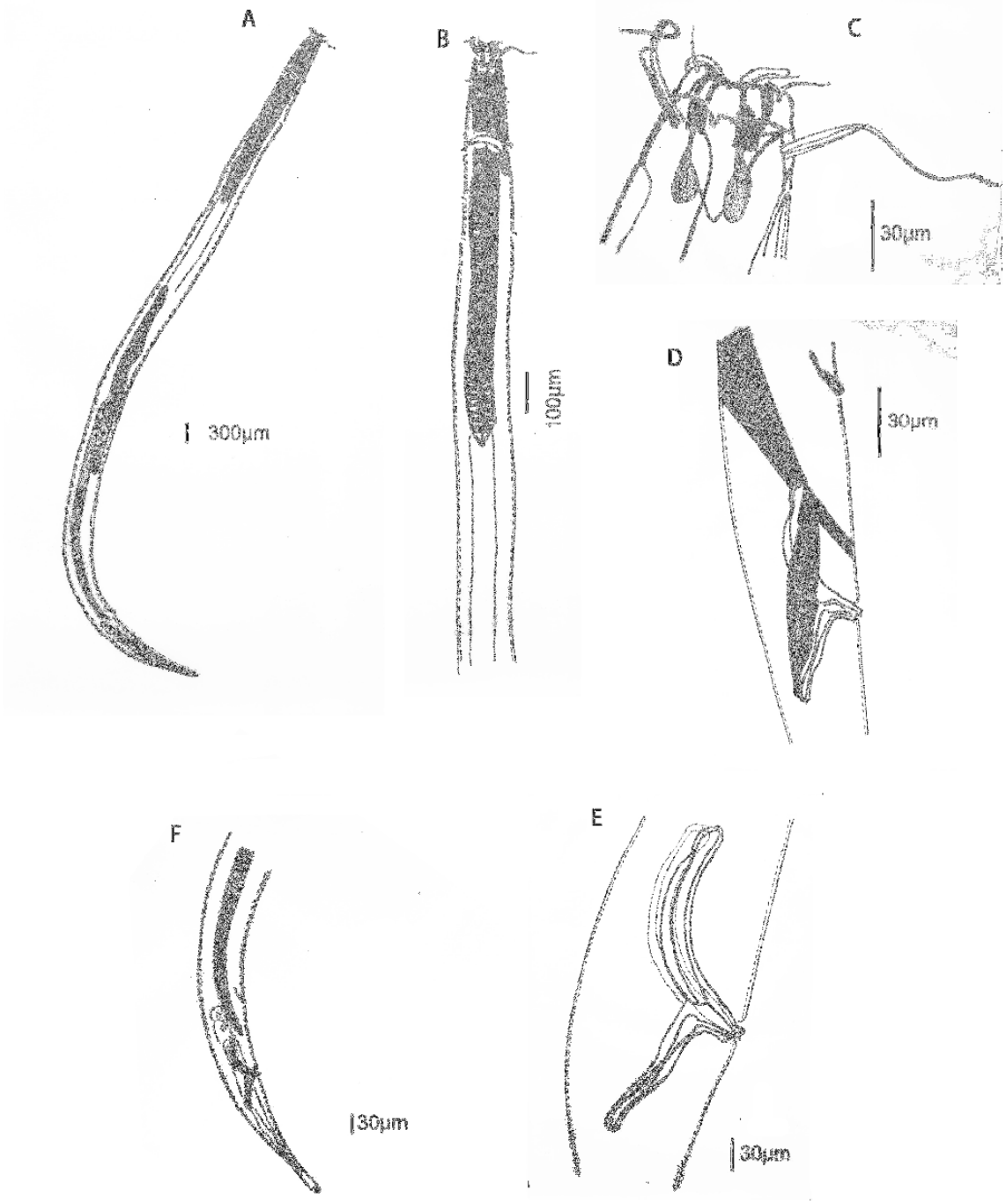

Figure 12. Male. *Mesacanthion dentantuspiculum* sp. nov. A. Aspect général du corps, B. Anterior region with the esophageal glands, C. Head and the buccal cavity with mandibles and teeth, D. Spicules and supplements, E. Detail of spicules, F. supplements, spicules, and tail.

Figure 12. Mâle. *Mesacanthion dentantuspiculum* sp. nov. A. Total body, B. Région antérieure avec glandes oesophagiques, C. Tête avec cavité bucale avec mandibules et dent, D. Détail des spicules, E. Détail des spicules, F. Supplément, spicules et queue.

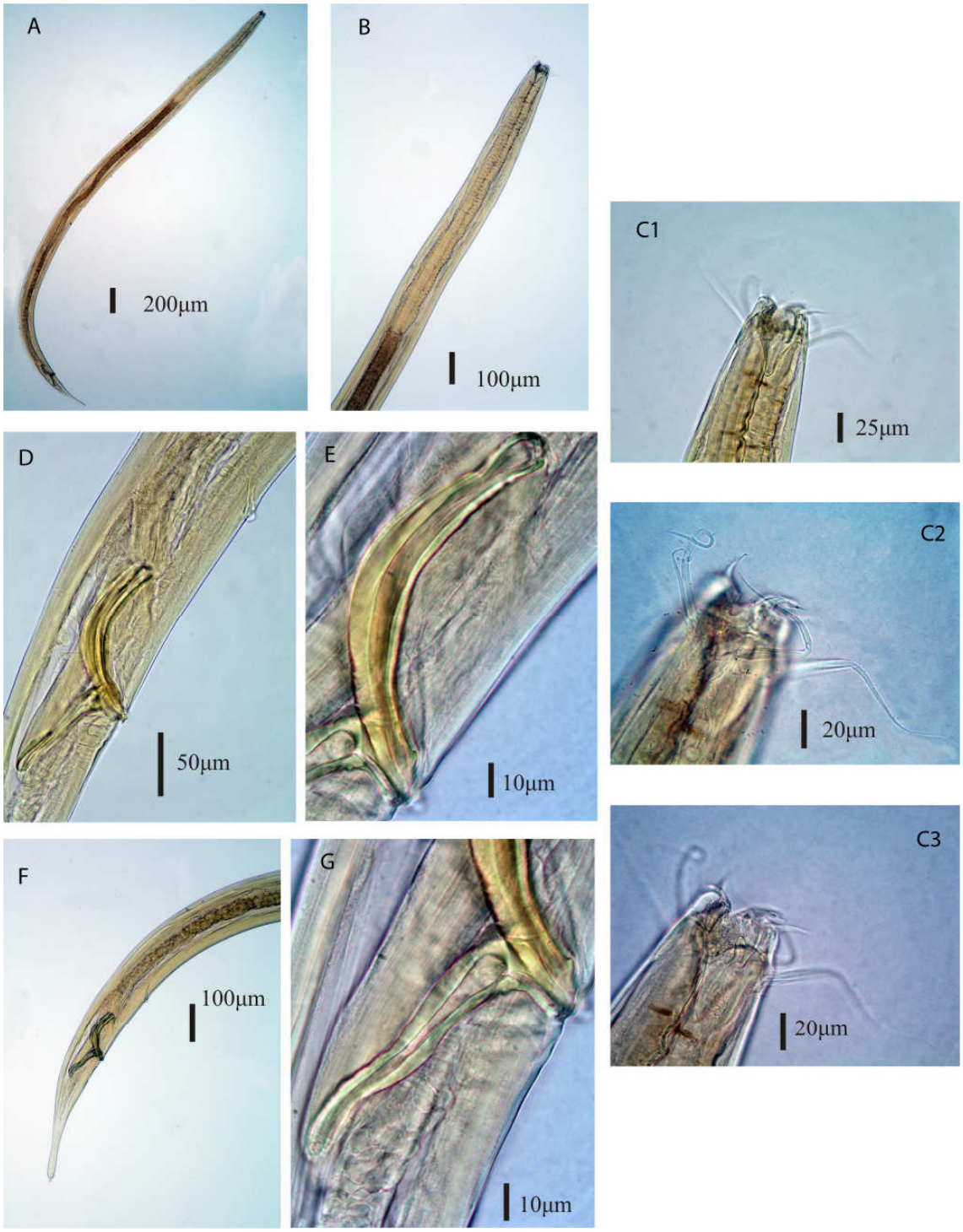

Figure 13. Male. *Mesacanthion dentantuspiculum* sp. nov. A. Total body, B. Mandible detail, C1, C2 and C3. Head and the buccal cavity with mandibles and teeth, D. Spicules and supplements, E. Detail of spicules, F. supplements, spicules, and tail, G. Detail of gubernaculum.

Figure 13. Mâle. *Mesacanthion dentantuspiculum* sp. nov. A. Aspect général du corps B. Détail et mandibules, C1, C2 et C3. Tête avec cavité bucale avec mandibules et dent, D. Détail spicules et supplements, E. detail et spicules, F. supplément, spicules et queue, G. Détail du gubernaculums.

Discussion

The genus *Mesacanthion* belongs to the subfamily Enoplolaiminae De Coninck, 1965 mainly in having the buccal cavity with mandibles and three teeth.

Mesacanthion henriquei sp. nov. possesses the general features of the genus, but the specific character is the combination of the cephalic setae shorter than the outer labial, however in different circles; presence of onchia connected to the mandibles, cervical glands, testes in tandem and spicule-shaped. This kind of testes, well described by Warwick *et al.* (1998), has not yet been described for the genus *Mesacanthion*. According to Lorenzen (1994), in general, the family Thoracostomopsidae possesses two opposed testes, but he did not furnish details of the position and shape.

This new species possesses long striated spicules. The following species have long spicules without striations: *M. audax, M. cavei, M. ceeum, M. gracilisetosus, M. infantile, M. kerguelense, M. longispiculum,* and *M. southerni. M. diplechma, M. audax, M. infantile,* and *M. kerguelense* also differ from the new species in possessing a gubernaculum with apophysis.

The valid species *M. africanum*, *M. diplechma*; *M. obscurum,* and *M. southerni* are similar to *M. henriquei* sp. nov. in possessing both long and striated spicules; however, they differ in having anisomorphic and anisometric spicules. Among the valid species of the genus *Mesacanthion* from marine and freshwater environments, five species have males with anisomorphic and anisometric spicules: *M. diplechma, M. africanum, M. southerni, M. obscurum* and *M. propinquum* (Gerlach & Riemann, 1974; Boucher, 1977; Nicholas, 1993).

According to Wieser (1953), the females of the species of *Mesacanthion* are separated in two groups, with asymmetrical or symmetrical ovaries. The female of *M. henriques* sp. nov. belongs to the group with symmetrical ovaries. The only female that is known to possess asymmetrical ovaries belongs to the species *M. lucifer*.

Mesacanthion dentantuspiculum sp. nov. presents the same general features described previously for the genus *Mesacanthion*, but has a different shape and length of the spicule. *M. africanthiforme, M. infantilis, M. longissimesetosum, M. majus, M. monhystera, M. pacificum, M. proximum,* and *M. studiosum* all resemble *M. dentantuspiculum* sp. nov. in possessing short spicules. However, they differ from the new species in having denticles in the proximal, distal, ventral and dorsal regions, and in having a bifid distal region. *Mesacanthion audax,* described by Ditlevsen (1918), has spines on the distal portion of the spicules, but only on the tip.

The diagnostic features of this new species consist of morphometric differences and the shape and length of the spicules.

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CAPÍTULO 03

Description of a new species of *Epacanthion* (Thoracostomopsidae, Nematoda) from Brazil and a modified key for species identification.

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Description of a new species of *Epacanthion* (Thoracostomopsidae, Nematoda) from Brazil and a modified key for species identification*

BETÂNIA CRISTINA GUILHERME¹, MARIA CRISTINA DA SILVA & ANDRÉ MORGADO ESTEVES

Laboratório de Meiofauna, Universidade Federal de Pernambuco, Rua Prof. Moraes Rego, 1235, Cidade Universitária, Recife-PE, CEP 50670-901.

¹Corresponding author: betania.bianne@yahoo.com.br

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Abstract

Epacanthion aguiberna culus sp. nov. (Thoracostomopsidae, Nematoda) is described from sediments of Campos Basin, Atlantic Southeast, Brazil. The main features are: the long spicules, the absence of gubernaculum, and the presence of three pairs of setae in the tail region. Only one male was found for description, but the main features were strong enough to consider it clearly as a new species. An updated and modified key for species identification is proposed.

Key words: free-living marine nematodes, deep sea, Campos Basin

Introduction

The family Thoracostomopsidae Filipjev, 1927 belongs to Enoplida because of the presence of a smooth cuticle, metanemes, and a non-spiral amphideal fovea. This family is composed of three subfamilies: Thoracostomopsinae Filipjev, 1927, Trileptiinae Gerlach & Riemann, 1974 and Enoplolaiminae De Coninck, 1965 (Lorenzen 1994). The differential features of the subfamilies consist of the presence or absence of teeth and mandibles in the buccal cavity, except that members of Thoracostomopsinae bear a long and eversible spear (Smol & Coomans 2006).

There are few recent taxonomic studies on the Thoracostomopsidae (Lorenzen 1981; Greenslade & Nicholas 1991). Lorenzen (1981) listed 16 genera belonging to this family. Greenslade & Nicholas (1991) revised the family, described two new genera from Australia, and recognized 21 valid species of *Epacanthion* Wieser, 1953 (Wieser 1953a).

According to Mawson (1956), the main differential feature of *Epacanthion* is the structure of the mandible. However, Gerlach (1956) considered the structures of the head to be more robust features for the genus diagnosis. In the most recent revision of this genus, Greenslade & Nicholas (1991) provided an identification key following the proposals of Mawson (1956) and Gerlach (1956), and also utilized the features of the male reproductive system, such as spicules and gubernaculum.

The genus *Hyalocanthion* Wieser, 1959 is generally similar to *Epacanthion*, so Inglis (1966) proposed it to be a synonym of *Epacanthion* because of the similarity in the mandible structures.

The 21 valid species of *Epacanthion* have the cephalic capsule strongly sclerotized, cephalic setae inserted slightly above the cephalic capsule, three teeth present, and three strongly sclerotized mandibles that are formed by two solid longitudinal columns, united by a thin membrane. The structure of the mandibles is the most important feature for the genus diagnosis. According to Nicholas & Greenslade (1991), *Epacanthion*

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is closely related to *Enoplolaes* Stekhejev, 1912 and *Metacanthion* Filipjev,1927, but differs from these two genera by the absence of a solid connection between the mandibular bars. Wieser (1953b) described the mandibles of *Epacanthion* as intermediate in structure between those of *Enoplolaes* and *Metacanthion*.

In a previous evaluation of material collected in the Campos Basin, members of two genera of the family Thoracostomopsidae were found: *Metacanthion* and *Epacanthion*. A new species of the latter genus is described here.

Material and Methods

The Campos Basin is located on the continental shelf and slope off Rio de Janeiro, Brazil, between 21° 30 and 23°30' S. The area and the field methodology are described by Botelho *et al.* (2007).

Nematodes were extracted by manual elutriation, as described by Boisseau (1957). After the sorting process, the nematodes were slowly transferred to glycerin (De Grisse 1969). The methodology described by Cobb (1917) was used to mount the permanent slides. Measurements and drawings were made with an OLYMPUS CX31 optical microscope, with the aid of a drawing tube. Photographs were taken with a C-5050ZOOM Olympus digital camera.

The holotype was deposited at the National Museum of Rio de Janeiro (MNRJ), Brazil.

Abbreviations used in the text:

abd: anal body diameter
cbd: corresponding body diameter
L: body length
mbd: maximum body diameter
ph: length of pharynx
t: tail length
a: L/mbd
b: L/ph
c: L/t
c': t/abd

The name of the body regions followed Coomans (1979). All the measurements are expressed in micrometers. All curves are measured along the cord.

Taxonomy [after Smol and Coomans (2006)]

Enoplida Chitwood & Chitwood,1937
Enoplina Chitwood & Chitwood,1937
Enoploidea Dujardin,1845
Thoracostomopsidae Filipjev, 1927
Enoplolaiminae De Coninck, 1965
***Epacanthion* Wieser, 1953**

Diagnosis of Thoracostomopsidae (according to Smol & Coomans 2006). Lips high. Only dorsolateral orthometanemes with robust scapulae but no caudal filament. Inner labial sensilla robust and setiform (papilliform only in *Penestrolaimus* Filipjev,1927), outer labial and cephalic setae robust and long. Epidermal glands with particularly well-differentiated outlet. Inner layer of cuticle forms a gland with particularly well-differentiated outlet. Inner layer of cuticle forms a cephalic capsule on to which pharyngeal muscles are attached. Cephalic organs often present and of variable shape. Amphids small and situated posterior to the

cephalic capsule or absent. Spacious buccal cavity with three mandibles and three teeth (mediodorsal and two ventrosublateral) or with one long eversible spear. Female reproductive system didelphic–amphidelphic with antidromously reflexed ovaries (a single posterior ovary in *Mesacanthion monhystera* Gerlach, 1957). Caudal glands penetrate into the precaudal region.

Diagnosis of Epacanthion Wieser, 1953 [according to Smol & Coomans (2006)]. Enoplolaiminae. Cuticle usually smooth. Head broadly wedge- or cone-shaped. Lips high, mostly striated. Inner labial setae long and inserted at the base of lip flaps; outer labial and cephalic setae situated at middle or anterior end of cephalic capsule. Cervical setae often present, can be numerous in males, and are sexually dimorphic. Mandibles consist of two plate-shaped columns separated by a thin sheet of cuticle (space between columns not solid) and only connected anteriorly by a bar (an intermediate stage between *Enoploides* and *Mesacanthion*); mandibular teeth small with gland which opens at tip. Pharynx relatively long and cylindrical; cardia pyriform. Females didelphic–amphidelphic with reflexed ovaries at left side of the intestine. Males diorchic with both testes at left side of the intestine; gubernaculum without apophyses present or absent. Spicules mostly long (≥2.5 anal diameters long) or short; gubernaculum without apophysis present or absent. Preanal supplement present or absent. Three caudal glands, cells pre-caudally. Tail narrowly conical or attenuated.

According to Greenslade & Nicholas (1991), there are 21 valid species:

- *Epacanthion brevipiculatum* Mawson, 1958
- *Epacanthion brevipiculum* Mawson, 1956
- *Epacanthion hitchiti* (Southern, 1914)
- *Epacanthion dubopalle* (Kreis, 1929)
- *Epacanthion enoploidiforme* (Gerlach, 1953)
- *Epacanthion exploratoris* Greenslade & Nicholas, 1991
- *Epacanthion flagellicaudum* Gerlach, 1956
- *Epacanthion galeatum* Boucher, 1977
- *Epacanthion georgei* Inglis, 1971
- *Epacanthion gorgonocephalum* Warwick, 1970
- *Epacanthion marioni* Warwick, 1977
- *Epacanthion microdentatum* Wieser, 1953
- *Epacanthion multipapillatum* (Wieser, 1959)
- *Epacanthion murmanicum* (Saweljev, 1912)
- *Epacanthion nadjae* Sergeeva, 1974
- *Epacanthion oliffi* Inglis, 1966
- *Epacanthion oweni* Keppner, 1986
- *Epacanthion pellucidum* (Saweljev, 1912)
- *Epacanthion polyastomum* (Jensen, 1986)
- *Epacanthion saveljevi* (Filipjev, 1927)
- *Epacanthion stekhoveni* Greenslade & Nicholas, 1991

***Epacanthion agudarmaculus* sp. nov.**

Material studied: One male.

Type material: Holotype MNRJ 329, Station 53 (Figure 1).

Type locality: Southern part of Campos Basin (Rio de Janeiro, Brazil) at 1950 m depth, in silt-clay sediments.

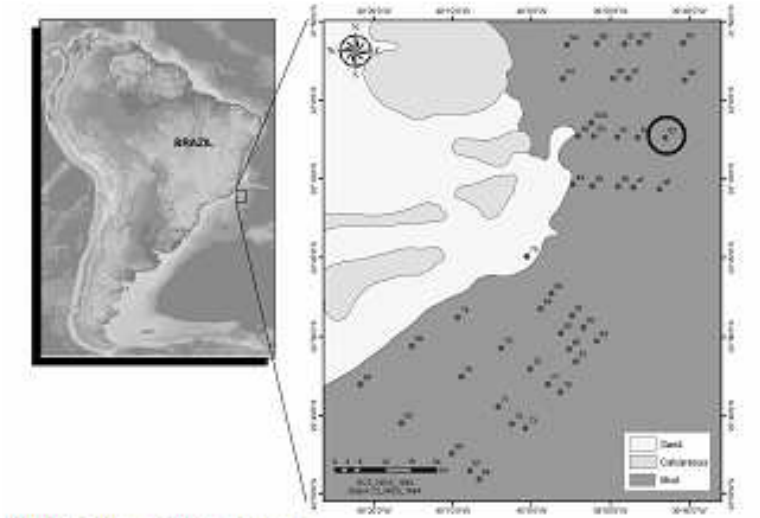

FIGURE 1. Study area showing sampling stations.

Etymology. The Latin "agubernaculus" refers to the absence of a gubernaculum (a = without + gubernaculus = gubernaculum).

Description (Holotype). (Figs. 2 and 3) Cylindrical body (L=2045.2 μm) tapering towards both extremities. Smooth cuticle. Cephalic capsule strongly sclerotized (43.2 μm). Sensilla arranged in two circles (6+10): six inner labial sensa (10.8 μm), six outer labial sensa (31.7 μm) and four cephalic sensa (11.4 μm) in the second row. The second circle is inserted slightly above the base of the cephalic capsule (3.6 μm apart). Several setae of varying lengths present in the cervical region. Somatic setae (35.4 μm) on the body, with no distinct pattern. Highlips composed of three strongly sclerotized mandibles (20.4 μm long and 4 μm wide) of two longitudinal solid bars united by a thin membrane, mandibles curved in the base of the buccal cavity where three equal teeth are found (4.8 μm long and 20.4 μm wide), one dorsal and two ventrosublateral. One gland present in the base of each tooth. Amphidial fovea indistinct. Pharynx strongly muscular (516 μm long), corresponding to 87.6% of the anterior region. Nerve ring (55.13 μm) located forward of the middle portion of the pharynx (178.5 μm) and occupying 67.3% of the corresponding body diameter. Cardia triangular. Reproductive system dioichic, testes located at the left of the intestine (in tandem), extending to the anterior region of the body, anterior testis 233.5 μm long and posterior testis 171.1 μm long. Spicules long (186 μm), transversely striated with four points of sclerotization concentrated in the proximal portion and in the middle of the spicules. The distance between the sclerotization points ranged from 79 μm to 121 μm. Gubernaculum absent. One tubular precloacal supplement, finely sclerotized (18 μm long and 1.8 μm wide) and placed near the proximal region of the spicules, 104.4 μm distant from the cloaca. Tail conico-cylindrical (139.2 μm long). Three pairs of setae (range 3.6–13.8 μm long) in the conical portion, no setae in the cylindrical tail region. Caudal glands not observed.

L= 2045.2; a= 25.18; b=3.9; c=14.6 ; c'=2.7

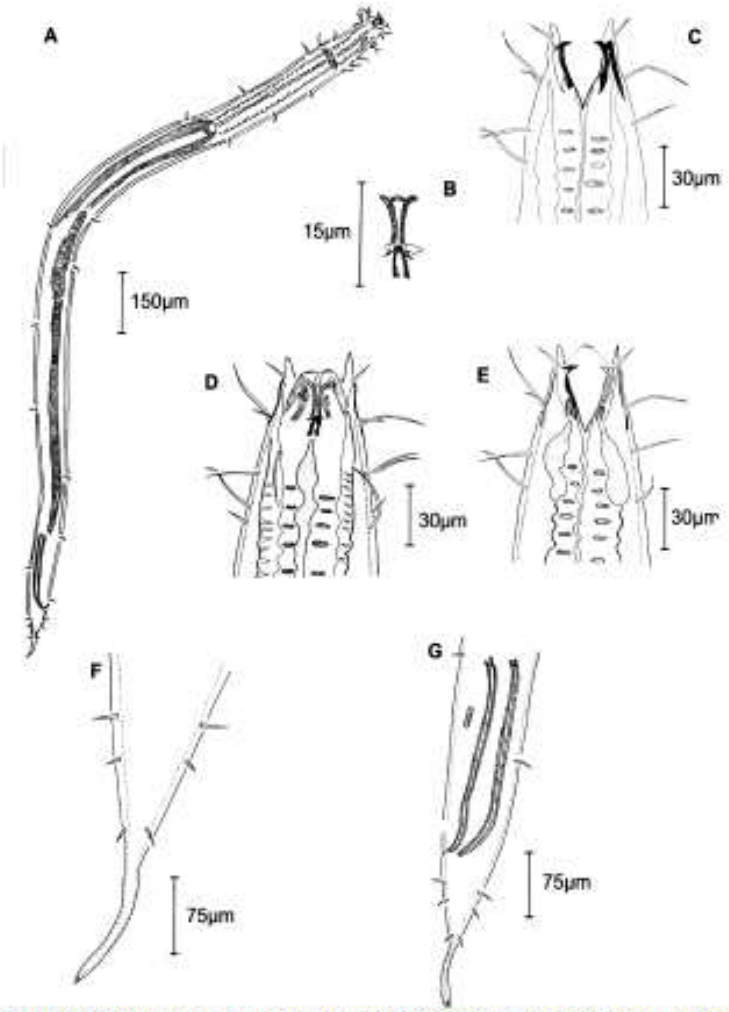

FIGURE 2. Male. A.Total body, B. Mandible detail, C. Head and the buccal cavity with mandibles,D. Anterior region, mandibles and Teeth, E. Anterior region with the esophageal glands, F. Setae in the tail region, G. Spicules, tail and supplements.

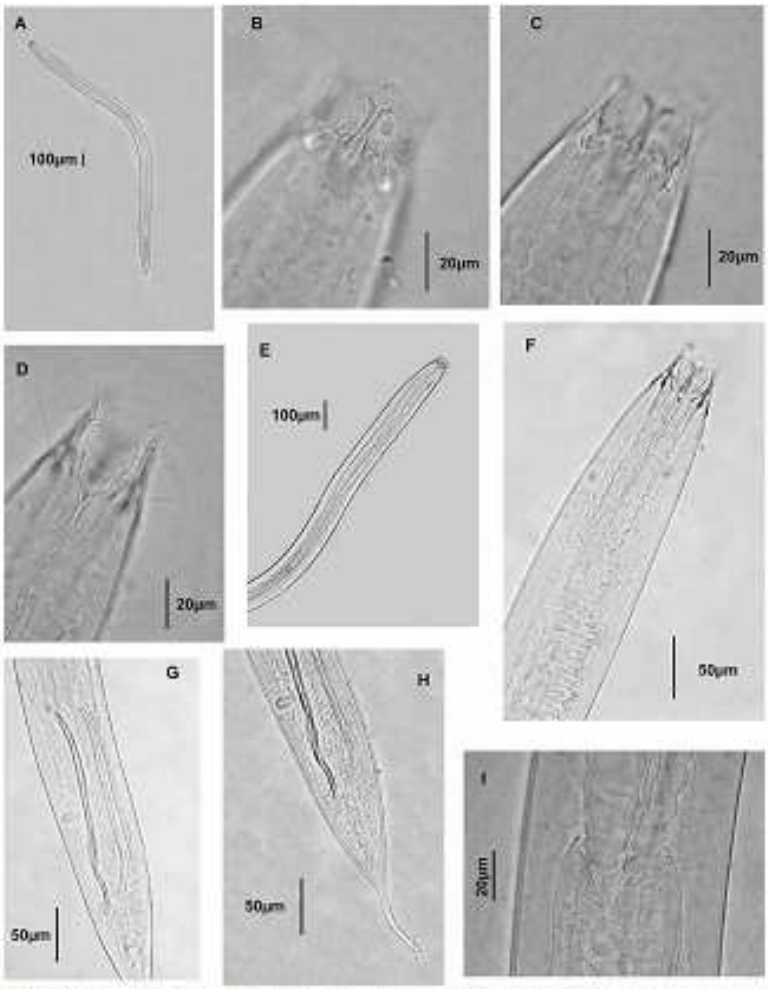

FIGURE 3. Male. A.Total body, B. Mandibles,C. Teeth (Mandibles),D. Buccal cavity, E. Pharynx region,F. Somatic setae,G. Spicules,H. Tail, I. Cardia region.

Discussion

The genus *Epacanthion* belongs to the subfamily Enoplolaiminae De Coninck, 1965, mainly because of the presence of three mandibles and three teeth in the buccal cavity. According to Smol & Coomans (2006), this subfamily needs to be revised because of the complex structure of the buccal cavity as well as deficiencies in published descriptions of the cephalic organs. The function of these organs is not well understood, although some attempts to explain their role have been made. For example, Wieser (1953c) suggested a possible relationship between the function of this structure and the predatory activity of nematodes, as it has a powerful buccal armature. In this study, as well as nearly all of the species descriptions for *Epacanthion*, the cephalic organs could not be observed. For other genera, such as *Paramarcanthion* and *Enoploides*, few descriptions include the observation and position of the cephalic organs in the cephalic capsule (Wieser 1953b; Warwick 1970; Bussau 1995). The reason for the neglect of this complex structure in both older and modern species descriptions may be due to the difficulty in adequately observing it.

The new species is similar to *Epacanthion hitschii* and *E. durapelle* with respect to one pharyngeal gland opening through each tooth, the shape of the spicules, and the position of the precloacal supplement. The three species differ in the lengths of the spicules: in *E. hitschii* and *E durapelle* the spicules are longer than 200 µm, whereas the new species has small spicules 185 µm long.

E. agubernaculus sp. nov. shows the same arrangement of labial and cephalic setae as found in *E. hitschii*, *E. anoploidiforme*, *E. gorgonacephalum*, *E. multipapillatum*, *E. pellucidum*, and *E. polysetosum*. However, the setae differ in length among the species.

Currently, the diagnosis of *Epacanthion* includes species with both short and long spicules, but in the past the length of the spicules was considered one of the most differential attributes (Wieser 1959). Wieser (1959) determined that only specimens of spicules longer than 2.5 µm should be included in the genus. Only the new species, *E. brevispiculatum*, *E. hitschii*, and *E durapelle* have spicules in this category. However, other species presently ascribed to this genus, such as *E. brevispiculum*, have spicules smaller than the limit proposed by Wieser (1959).

Even though *E. agubernaculus* sp. nov. belongs to the group of species with long spicules, we consider the absence of a gubernaculum to be a distinctive feature in the species of this group. This is also a characteristic shared with *E. oliffi*, a species with short spicules.

The Brazilian species also has three pairs of setae in the conical region of the tail, and the pairing of setae is now reported for the first time among the species of *Epacanthion*.

Although the description of *E. agubernaculus* sp. nov. is based on a single specimen, several morphological features, such as the absence of a gubernaculum and the pairing of caudal setae in the anterior region, are sufficient to establish it as a new species.

Key to species of *Epacanthion*

1	Spicules without gubernaculum 2
-	Spicules with gubernaculum 3
2	Spicules long (more 100 µm), one pre-anal supplement ..... *E. agubernaculus*
-	Spicules short (less 100µm), without pre-anal supplement ..... *E. oliffi*
3	Spicules short, 2-5 or fewer anal diameters long 4
-	Spicules long, 3 or more anal diameters long 14
4	Pre-anal supplement present 5
-	Pre-anal supplement absent 12
5	Nine to 14 small, setose pre-anal supplements 6
-	Only one pre-anal supplement 7
6	Distance between tips of apical teeth about equal to length of mandibular columns, which are short, stout and parallel ..... *E. multipapillatum*
-	Mandible with tips of apical teeth much closer together than length of mandibular columns, which are slender and divergent at apex ..... *E. evansi*

7 Distance between tip of apical mandibular hooks either clearly much greater or much less than length of mandibular columns 8
- Distance between apical mandibular hooks apparently about same as length of mandibular columns ..... *E. brevispiculum*
8 Distance between tip apical mandibular hooks much greater than length of mandibular columns 9
- Distance between apical mandibular hooks much less than length of mandibular columns 10
9 Spicules 1–2 anal diameters (59 μm), gubernaculum a triangular plate, dorsal cirrhus much smaller than other two, 12 pairs of cervical setae in male, 4 in female ..... *E. mawsoni*
- Spicules 0–7.5 anal diameters (34 μm), gubernaculum reduced, cirrhus apparently unequal, male with 12 cervical setae (female unknown) ..... *E. pallidulum*
10 Spicules 2–8 anal diameters (80 μm), gubernaculum present (37 μm) lip not striated ..... *E. microdentatum*
- Spicules 1 anal diameter (90 μm), lip flagellated 11
11 Small species (1.5–2.0 mm long), male head hirsute, cephalic setae maximum length 30 μm, inserted above cephalic arch, posterior rim of cephalic capsule crenulated (inflation) ..... *E. galeatum*
- Larger species (2.0–2.9 mm long) ,male head hirsute, cephalic setae maximum length 50 μm, longer than head diameter,inserted just below cephalic arch, posterior rim of cephalic capsule not crenulated ..... *E. exploratoris*
12 Male head hirsute with many subcephalic and cervical setae ..... *E. aviplidiformis*
- Male head not hirsute 13
13 Mandibles very long and slender, mandibular columns about twice as long as distance between apical teeth ..... *E. gorgonocephalum*
- Mandibles not long and slender, distance between mandibular teeth less, or only slightly longer (1.2x), than mandibular columns ..... *E. georgei*
14 Tail long, 4 or more anal diameters, spicules of equal length, may be annulated 15
- Tail short, only 3 anal diameters ,spicules always annulated, may be of unequal length 18
15 Spicules >200 μm (3–3. 5 anal diameters) ,always annulated 16
- Spicules 130–175 μm (3–3. 5 anal diameters) long, not annulated 19
16 Larger species, males and females more than 4 mm long, a value 27–34, cirrhus nearly reaching cephalic arch, inner cephalic setae long, 40 μm 17
- Smaller species, 2.0–3.1 mm long, a value 11.7–19, cirrhus small, not reaching cephalic arch, inner cephalic setae very short, 26 μm ..... *E. dorapalla*
17 Tail setae numerous (>10 setae) ..... *E. huntschli*
- Tail setae sparse,(only two medial setae) ..... *E. stekhoveni*
18 Spicules of unequal ..... *E. nudjae*
- Spicules equal in length ..... *E. saveljevi*
19 Head blunt anteriorly, mandibular columns parallel, diverging strongly at apex, male supplement 90 μm in front of anus ..... *E. brevispiculatum*
- Head pointed anteriorly, mandibular columns parallel, male supplement 132–158 μm in front of anus ..... *E. polyaristatum*

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CAPÍTULO 04

Caracterização protéica de Nematoda de vida livre em dois ambientes distintos (marinho e estuarino)

Caracterização protéica de Nematoda de vida livre em dois ambientes costeiros.

Betânia Cristina Guilherme[1,2] Elba Verônica Matoso Maciel de Carvalho[3] , Luana Cassandra Breitenbach Barroso Coelho[3,] & Maria Tereza dos Santos Correa[3]

[1] Dept. Zoologia – CCB – UFPE – Cidade Universitária - Recife – Brasil - CEP 50670-420.
[2] Universidade Federal de Campina Grande – Olho d'água da Bica – Cuité - Paraíba – Brasil - CEP 58175-000. E-mail: betaguilherme@yahoo.com.br
[3] Dept. Bioquímica – CCB – UFPE – Cidade Universitária - Recife – Brasil - CEP 50670-420.

RESUMO

Os Nematoda de vida-livre são os animais mais abundantes dos sedimentos marinhos e estuarinos. Na sua composição orgânica as proteínas representam a maior classe bioquímica. Este estudo teve como objetivo levantar a composição protéica da nematofauna em dois ambientes costeiros e investigar o efeito do formol na composição protéica destes organismos. Amostras biosedimentológicos foram coletadas na praia de Maracaípe e Estuário do Pina com nove réplicas que foram acondicionados em potes de plásticos, contendo três tratamentos: (1) fixado com formalina neutra a 4% (S1), (2) formol a 4% tamponada com bórax (tetraborato de sódio) (S2) e (3) sem formol (S3). O tratamento sem formol (S3) foi mantido a 4ºC. Após a coleta, 200 nematódeos vivos foram extraídos por réplica e mantidos resfriados 20°C e posteriormente, congelados até a análise bioquímica. Os nematódeos conservados em formol foram extraídos três semanas. As amostras foram elutriadas (mínimo de 10 vezes) e vertidas em peneiras de 0,045mm para a extração da nematofauna. Os Nematoda foram sonicados e centrifugados para análise protéica usando método BCA (Protein Assay Kit). Para a determinação do peso molecular foi realizada uma eletroforese em gel de poliacrilamida contendo sulfato sódico de dodecila (SDS-PAGE) a 12 %. Nos ambientes estudados houve uma variação na concentração dos extratos obtidos dos organismos fixados com formalina neutra e os dos organismos tamponados com bórax, porém não significativa. Entretanto, para os extratos obtidos dos organismos sem tratamento a concentração de S1 e S2 foi cerca de 78% menor. Uma variação significativa, ocorreu para os extratos obtidos dos Nematoda coletados no complexo estuarino da Bacia do Pina- PE sem formol, onde a concentração protéica foi de 80,27 µg/mL, cerca de 68% maior comparado com S3 da praia de Maracaípe. Há a necessidade de mais estudos a cerca da composição protéica dos nematódeos marinhos e estuarinos e os primeiros resultados aqui apresentados, demonstraram existir uma variação considerável entre os ambientes, logo, um estudo mais detalhado, iria responder com mais eficácia essas dados protéicos.

Palavras-chave: Nematofauna, BCA, Praia de Maracaípe, Estuário do Pina.

ABSTRACT

The free living nematodes are the animals more abundant into the marine and estuarine sediments. In their organic composition, the proteins represent the major biochemistry class. Although, this study had the aim that identify the protein composition of the nematofauna in two coastal environmental and to investigate the effect of formalin in the organisms. Sediments samples were collected in Maracaípe beach and Pina estuary and nine replicates were pick out and preserved with three treatments: (1) neuter formalin 4% (S1), (2)

formalin 4% buffered with sodium tetraborate (S2) and (3) without formalin (S3). The treatment without formalin (S3) was maintained at 4ºC. After the sampling, were extracted at least 200 nematodes in each replicate and kept at 20°C during all process and posteriorly frozen for biochemistry analysis. The nematodes preserved with formalin were extracted in the same quantity after the period of three weeks. The sediment samples were elutriated, at least 10 times and washed in mesh sieve size of 0,045mm for extraction of nematodes. For each treatment, the nematodes were pick out, sonicated and centrifugated for protein analysis using the BCA methods (Protein Assay Kit). The determination of molecular weight was realized in polyacrilamide gel in presence of sodium dodecyl sulphate (SDS-PAGE) at 12 %.
In the studied atmospheres there was a variation in the concentration of the obtained extracts of the organisms fastened with neutral formalina and the one of the organisms tamponados with borax, however no significant. However, for the obtained extracts of the organisms without treatment the concentration of S1 and S2 was about 78% smaller. A significant variation, happened for the obtained extracts of Nematoda collected in the compound estuarine of the Basin of Pina - FOOT without formol, where the concentration protéica was of 80,27 µg/mL, about 68% adult compared with S3 of the beach of Maracaípe. The first results presented here demonstrated that exist a variation between the two habitats (Pina and Maracaípe), however it is necessary more research about protein composition of the marine and estuarine nematodes because a study with more details could answer efficient these protein data.

Key-words: Nematofauna, BCA, Maracaípe beach, Pina estuary.

INTRODUÇÃO

Os Nematoda de vida-livre são os animais mais abundantes dos sedimentos marinhos e estuarinos (GIERE, 2009), considerados como componentes mais representativos dos metazoários (VINCX *et al*., 1994, MONTAGNA *et al*.,1995). Os registros quanto ao número de espécies de Nematoda diferem muito, existindo mais de 20.000 espécies de vida livre descritas, com a maioria destas vivendo em ambientes marinhos (EYUALEM-ABEBE *et al*., 2008). Nos sedimentos marinhos, estes organismos podem representar de 80 a 95% dos indivíduos e de 50 a 90%, da biomassa, do meiobentos (GIERE, 2009). Esses animais podem ser utilizados como bioindicadores em avaliações de biodiversidade e biomonitoramento (LAMBSHEAD, 2004; YEATES & BOAG, 2004; COOK *et al*., 2005).

Os Nematoda são considerados uma ferramenta importante e promissora em estudos de poluição devido à sua distribuição, que abrange praticamente todo tipo de ecossistema, e à sua elevada diversidade taxonômica (HODDA & NICHOLAS, 1986) possuindo, conseqüentemente, uma sensibilidade a diversos poluentes e alterações ambientais (BONGERS & FERRIS, 1999). Diferenças nos parâmetros abióticos podem estar representadas em dois ambientes costeiros e também ocorrer dentro de um mesmo ambiente,

como resultado de alterações naturais e/ou antrópicas. Estas variações podem levar a modificações na estrutura da comunidade em termos de distribuição, abundância, diversidade dos organismos (GIERE, 2009) e provavelmente da composição protéica destes organismos.

De acordo com Danovaro *et al*. (1999) as proteínas representam a maior classe bioquímica de compostos orgânicos em Nematoda. Através de análise em eletroforese bi-dimensional de extratos de estágios misturados de *C. elegans* revelaram pontos de gel corados em prata, representando 2000 proteínas diferentes dentro PI que variam entre 3,5-9 e o peso molecular variando de 10-200 kDa (BINI *et al.*, 1997). É estimado que em media a célula eucariótica expresse 05 a 10000 proteínas diferentes. A dominância de proteínas também foi observada na maioria dos estudos, quando comparada à composição bioquímica de outros organismos marinhos, dentre estes: Copepoda (BAMSTEDT 1975, 1978); Copepoda Harpacticoida (MILIOU *et al*. 1992); misidáceos (BAMSTEDT, 1978); Eufausiáceos (VIRTUE *et al*., 1995) e Bivalvia (ANSELL, 1974).

Vários estudos já foram realizados com Nematoda, porém, os mesmos tendem a ser mais investigados na sua importância médica e veterinária, porque a maioria deles são pestes de culturas. Existe uma vasta literatura para nematódeos parasitas em relação a sua estrutura, sistemática, bioquímica, fisiológica, imunológica e biologia molecular (LEE, 2002). Estudos bioquímicos, envolvendo proteínas solúveis em Nematoda parasitas foram realizados nos últimos 30 anos (CARNEIRO & ALMEIDA, 2001). Entretanto, os de vida livre, já foram estudados com maior ênfase no ponto de vista ecológico, sistemático e molecular, mas estudos bioquímicos, principalmente protéicos, são raros. O estudo sobre a composição bioquímica de Nematoda de vida livre em mar profundo e praias arenosas, foi realizado por Danovaro *et al.*(1999) levando em consideração seu conteúdo protéico controlado por mudanças em condições ambientais e, em particular, por alterações na qualidade e na quantidade de alimentos disponíveis para o bentos.

Este estudo foi realizado para testar a hipótese de que (1) Existe uma variabilidade nos componentes protéicos e/ou de suas proporções na nematofauna em dois ambientes costeiros (marinho e estuarino); (2) os efeitos do formol sobre a determinação da composição em Nematoda de vida livre, com outros ambientes já estudados.

MATERIAIS E MÉTODOS

Coleta em campo

As amostras biosedimentológicas foram coletadas em dois ambientes costeiros distintos: na praia de Maracaípe (Figura 1) que se localiza no município de Ipojuca, litoral Sul de Pernambuco, Possui 7 km de extensão e é caracterizada por uma área exposta com inclinações médias de 5° e ondas quebrando próximo à praia ao Norte e uma área abrigada, com inclinações médias de 3° e protegida por recifes ao Sul, onde se localiza a foz do rio Maracaípe. O outro ambiente prospectado foi o complexo estuarino da Bacia do Pina (Figura 2), que está localizada na zona litorânea do Estado de Pernambuco, situando-se na parte interna do Porto do Recife-PE, entre os paralelos 08°04’03 e 08°05’06”S e os meridianos 34°52’16” e 34°53’58”W. Nos dois ambientes, as coletas do sedimento foram realizadas com um tubo PVC de 3,7 cm de diâmetro interno, sendo este inserido nos sedimentos até 10 cm de profundidade, no mediolitoral inferior. Para investigar o efeito do formol na composição protéica dos Nematoda nos diferentes ambientes, foram retiradas nove réplicas das amostras de sedimentos aleatórias e acondicionados em potes de plásticos, contendo três tratamentos: (1) fixado com formol neutro a 4% (S1), (2) formol a 4% tamponada com bórax (tetraborato de sódio) (S2) e (3) sem formol (S3) e (S3-E) designado para o estuário da Bacia do Pina. O tratamento que não continha formol (S3) foi mantido a 4ºC. Em seguida, todas as amostras foram transportadas para o Laboratório de Meiofauna do Departamento de Zoologia da UFPE. As amostras biosedimentológicas fixadas ou não, foram colocadas em Becker de 1.000 ml, lavadas e elutriadas sucessivamente (mínimo de 10 vezes). O sobrenadante resultante deste procedimento foi vertido em peneiras geológicas de 0,045mm para a extração da nematofauna. Após a coleta, os Nematoda vivos foram extraídos pelo menos 200 indivíduos por replica (n = 3 a 5) e mantidos resfriados 20°C durante todo o processo e posteriormente, congelados até a análise bioquímica. Os Nematoda conservados em formol foram extraídos na mesma quantidade acima citada, após três semanas.

Figura 1: Praia de Maracaípe - PE com localização da área de estudada (localização do ponto prospectado) Fonte: Google mapa.

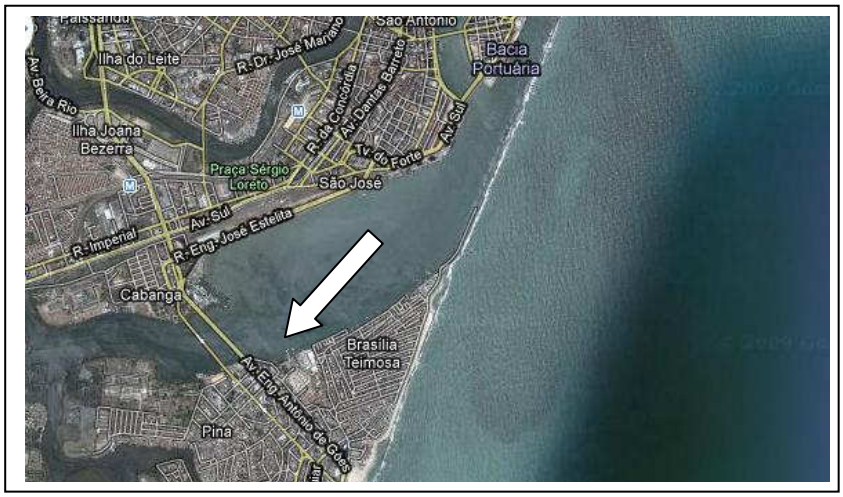

Figura 02: Bacia do Pina com localização da área de estudada (localização do ponto prospectado) Fonte: Google mapa

Preparação dos Extratos

Toda a vidraria utilizada para a composição bioquímica de nematódeos foi embebida em NaOH 1 M e posteriormente lavada com água Milli Q para evitar qualquer contaminação orgânica.

A fim de aumentar a eficiência da extração, antes de todas as análises, os nematódeos triados nos diferentes tratamentos, foram homogeneizados em 1 m1 de água MilliQ e depois

sonicados por 2h em 4ºC, congelados duas vezes por 30 minutos (-20 ° C) e sonicados novamente por 10 minutos a 4°C (DANOVARO *et al.,* 1999). Após esta etapa o material foi submetido centrifugação a 9.000.00 RPM a 4 ^{0}C por 15 minutos e os sobrenadantes armazenados a -16 °C para estudos posteriores.

Caracterização Bioquímica

Estimativa Protéica

A determinação quantitativa de proteínas foi efetuada de acordo com o método BCA (Bicinchoninic Acide - Pierce) de acordo com as normas do fabricante. Foi preparada uma curva padrão com albumina de soro bovino (BCA) com valores compreendidos entre 0 a 250 µg/ml e a leitura tanto da curva como das amostras, em tréplicas, foram realizadas a 590 nm.

Eletroforese em Gel de Poliacrilamida em Condição Desnaturante (SDS)

Foi realizada eletroforese em gel de poliacrilamida contendo sulfato sódico de dodecila (SDS-PAGE) a 12 % de acordo com Laemmli (1970) em todos os tratamentos da praia de Maracaípe, porém, para o estuário da Bacia do Pina só foi analisado o tratamento que continha Nematoda sem fixador. As bandas protéicas foram detectadas por coloração de prata (BioRad) de acordo com as normas do fabricante. Utilizou-se o modelo padrão Amaresco (Miosina 212 KDA, β. Galactosidase 116 KDA, Fosforilase B 97,4 KDA, Albumina 66,2 KDA, Ovalbumina 45,0 KDA, Anidrase Carbónica 31,0 KDA, Inibidor de tripsina de soja 21,4, Lisozima 14,4 KDA), no qual foi isolado das outras amostras e corado com azul de Comassie brilhante 0,02 % (p/v) em ácido acético a 10 % (v/v).

RESULTADOS E DISCUSSÕES

Proteínas

Nos ambientes estudados houve uma variação na concentração dos extratos obtidos dos organismos fixados com formalina neutra e os dos organismos tamponados com bórax, porém não significativa. Entretanto, para os extratos obtidos dos organismos sem tratamento a concentração de S1 e S2 foi cerca de 78% menor (Tabela 1). Uma variação significativa, ocorreu para os extratos obtidos dos Nematoda coletados no complexo estuarino da Bacia do Pina- PE sem formol, onde a concentração protéica foi de 80,27 µg/mL, cerca de 68% maior

comparado com S3 da praia de Maracaípe (Tabela 2). Danovaro *et al.*(1999) encontraram resultados semelhantes, onde o teor de proteínas variou entre 127 µg/mL e 69 µg/mL para os extratos que continham Nematoda fixados com formol; porém, a concentração dos Nematoda mantidos à frescos houve uma variação entre 119 µg/mL e 42 µg/mL. A composição de proteínas contidas no corpo dos Nematoda pode variar tanto entre espécies do mesmo gênero e entre indivíduos da mesma espécie (GRAEVE *et al*., 1997; GALLAGHER & AMBROSE, 1998). Porém, assim como nos dados investigados por Danovaro *et al.*(1999), este estudo também determinou a composição protéica da comunidade de Nematoda e não de uma única espécie, uma vez que a coleta de um grande número de organismos da mesma espécie é praticamente impossível, só podendo ser realizadas a partir de cultivo, mas, as condições ambientais e tróficas são claramente diferentes daquelas *in situ*, logo, provavelmente esses fatores afetariam, em grande parte, as determinações bioquímicas destes organismos.

Tabela 1: Comparação da concentração protéica dos extratos obtidos em Nematoda coletados na praia de Maracaípe – PE, estuário da Bacia do Pina.

Tratamentos	**Maracaípe**	**Bacia do pina**	**Danovaro *et al*. (1999)**
	µg/mL	µg/mL	µg/mL
Sedimento + formol neutro (S1)	121,3	159,8	127
Sedimento + formol tamponado com bórax (S2)	108,3	106,6	69
Sedimento sem formol (S3) e (S3-E)	25,6	80,27	42

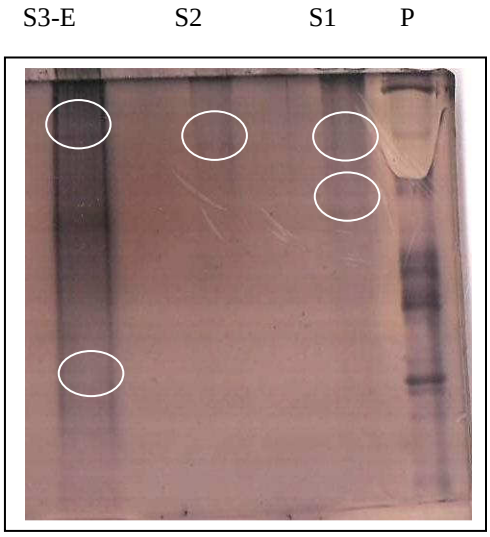

Figura 03: SDS (PAGE) dos tratamentos (S1 e S2) de Nematoda coletados na praia de Maracaípe-PE e S3-E no complexo estuarino da Bacia do Pina- PE. S1 (SEDIMENTO+FORMOL), S2 (SEDIMENTO+FORMOL/BÓRAX), S3-E (ESTUÁRIO) e P (PADRÃO)

Os perfis protéicos de bandas na SDS-Page apresentaram-se bastante diferentes. Os perfis da nematofauna em Maracaípe nos tratamentos S1 (sedimento+formol) e S2 (sedimento+formol/bórax), foram similares apresentando três bandas de alto peso molecular (97 KDA, 95 KDA E 91,6 KDA) e apenas o tratamento S1 evidenciou-se uma banda de peso molecular intermediário (74,7 KDA). Comparando os extratos S1 e S2 de praia com os do estuário do Pina (S3), observou-se perfis protéicos distintos, uma vez que em S3-E (estuário) foram observadas duas bandas principais, uma alto peso molecular (99 KDA) e outra de baixo peso molecular de (24,3 KDA), bem como várias bandas intermediárias (Figura 3). Vários estudos mostram dados relevantes sobre o perfil protéico de Nematoda. Em células germinativas de nematódeos *Graphidium strigosun* com numerosas bandas protéicas, porém, a maior apresentou peso molecular de 15 KDA, nas quais foram coradas com coomassie blue (MANSIN, 1999). Gaofu *et al*. (2008) avaliando a atividade lectínica de Nematoda parasitas de pinheiro, registram que o extrato purificado com proteínas LRA e PTA em géis de SDS-PAGE de poliacrilamida mostrou uma única faixa de proteína com um peso molecular acima de 12 kDa (variando entre 14.4 e 97.4, porém, em diferentes proporções nas diferentes proteínas). Estes estudos corroboram com as análises apresentadas por Bini *et al*. (1997) quando afirmam que o massa molecular em proteínas de Nematoda pode variar entre 10-200 kDa. Avaliando a concentração de proteínas em outro invertebrado, Moura *et al.* (2006) isolando lectinas de *Cliona varians*, observaram que as proteínas na ausência ß-mercaptoethanol, mostrou uma faixa com massa molecular de aproximadamente 106 kDa e as tratadas com ß-mercaptoethanol apresentaram quatro bandas com 28 kDa. Variações bioquímicas, principalmente concentrações de proteínas para invertebrados já foram registradas por BAMSTEDT (1975; 1978); MILIOU *et al*. (1992); VIRTUE *et al*. (1995) E ANSELL (1974).

No gel SDS-Page foi aplicada uma menor concentração protéica de Nematoda de estuário (S3-E), porém foi revelado, após coloração com prata, um maior número de bandas comparadas com os de praia. Isso, provavelmente revela que os diferentes perfis estejam correlacionados com as características tróficas em resposta a diferentes insumos de matéria orgânica.

Estudos realizados por Wakeham *et al*. (1993) e Chronis *et al.* (1996) testando os efeitos de diferentes tratamentos para preservar amostras de sedimento, demonstraram que, aplicando metodologias específicas, o uso de formalina como fixador não afeta a determinação de proteínas, carboidratos ou lipídios quando comparados às amostras não tratadas. Observações realizadas por Donovaro *et al.,* 1999, com Nematoda e utilizando-se a

mesma metodologia aplicada para este estudo, só que adicionaram rosa de bengala nas amostras com formol tamponado com bórax e em ambientes diferentes (mar profundo e costeiro), observaram que não há interferência dos mesmos nas análises em proteína e lipídios, e consequentemente na composição corporal dos Nematódeos. Segundo Saiz *et al.* (1998) a literatura sobre os efeitos de fixadores na composição bioquímica em material orgânica é extremamente escasso, o uso do formol como fixador pode causar graves problemas analíticos na determinação de certas classes de compostos orgânicos (tais como o carbono orgânico e de ácidos nucléicos), mas não em outros, como aminoácidos e lipídios. Portanto, estas afirmações corroboram com resultados encontrados nesse estudo, que a formalina não interferiu na composição protéica nos diferentes ambientes e que provavelmente essa expressão na quantidade de proteínas para os Nematoda de estuário, não pode ser até no momento explicado, pois outros fatores (abióticos e bióticos) devem ser levados em consideração. Entretanto, faz-se necessário um estudo mais detalhado, uma vez que as metodologias existentes que auxiliam a determinação de proteínas ou outras classes bioquímicas, são escassas, logo precisam ser ajustadas e adaptadas para organismos como Nematoda que apresentam um tamanho muito reduzido, dificultando assim a extração dos seus compostos orgânicos. Além disso, ainda que haja a necessidade de mais estudos a cerca da composição protéica dos nematódeos, os primeiros resultados aqui apresentados demonstraram ser esta abordagem, uma ferramenta promissora na investigação das estratégias bioenergéticas e na relevância trófica do grupo nos ecossistemas marinhos e estuarinos.

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4 CONCLUSÕES

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- As descrições das espécies da família Thoracostomopsidae são pobres, tanto no aspecto descritivo quanto no ilustrativo, sendo esse um fator limitante para o estudo dessa família. Assim, a primeira versão de uma atualização, aqui apresentada, dessa família facilitará a identificação de seus representantes;

- A realização do estudo taxonômico da família Thoracostomopsidae na Bacia de Campos e Bacia Potiguar revelou que, em alguns gêneros, existiam novas espécies para a Ciência. Assim a partir do material da Bacia de Campos, foi descrita uma nova espécie do gênero *Epacanthio*n. Para a Bacia Potiguar foram descritas duas novas espécies de *Mesacanthion* e uma nova espécie de *Epacanthion*.

- A partir do levantamento protéico da nematofauna nos ambientes estudados, pôde-se observar que houve uma variação considerável entre as concentrações de proteínas nos diferentes tratamentos. Porém, faz-se necessário um estudo mais detalhado sobre a mesma, uma vez que as metodologias existentes que auxiliam sua determinação são escassas. Desta forma, precisam ser ajustadas e adaptadas para organismos como Nematoda que apresentam um tamanho muito reduzido, dificultando assim, a extração dos seus compostos orgânicos.

- Ainda que haja a necessidade de mais estudos, acerca da composição protéica dos nematódeos, os primeiros resultados aqui apresentados demonstraram ser esta abordagem, uma ferramenta promissora na investigação das estratégias bioenergéticas e na relevância trófica do grupo nos ecossistemas marinhos e estuarinos.

5 ANEXOS

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ORIGINAL PAPER

Diversity of free-living marine nematodes (Enoplida) from Baja California assessed by integrative taxonomy

Tiago José Pereira · Gustavo Fonseca · Manuel Mundo-Ocampo · Betânia Cristina Guilherme · Axayácatl Rocha-Olivares

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Abstract We used morphological and molecular approaches to evaluate the diversity of free-living marine nematodes (order Enoplida) at four coastal sites in the Gulf of California and three on the Pacific coast of Baja California, Mexico. We identified 22 morphological species belonging to six families, of which Thoracostomopsidae and Oncholaimidae were the most diverse. The genus *Mesacanthion* (Thoracostomopsidae) was the most widespread and diverse. Five allopatric species, genetically and morphologically differentiated, were found in two localities in the Gulf of California (*M.* sp1 and *M.* sp2) and three in the Pacific coast (*M.* sp3, *M.* sp4 and *M.* sp5). Overall, we produced 19 and 20 sequences for the 18S and 28S genes, respectively. Neither gene displayed intraspecific polymorphisms, which allowed us to establish that some morphological variation was likely either ontogenetic or due to phenotypic plasticity. Although 18S and 28S phylogenies were topologically congruent (incongruence length difference test, $P > 0.05$), divergences between species were much higher in the 28S gene. Moreover, this gene possessed a stronger phylogenetic signal to resolve relationships involving *Rhabdodemania* and *Bathylaimus*. On the other hand, the close relationship of *Pareurystomina* (Enchelidiidae) with oncholaimids warrants further study. The 28S sequences (D2D3 domain) may be better suited for DNA barcoding of marine nematodes than those from the 18S rDNA, particularly for differentiating closely related or cryptic species. Finally, our results underline the relevance of adopting an integrative approach encompassing morphological and molecular analyses to improve the assessment of marine nematode diversity and advance their taxonomy.

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T. J. Pereira (✉) · A. Rocha-Olivares (✉)
Department of Biological Oceanography, Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Ensenada-Tijuana 3918, Apdo. Postal 2732, CP 22860 Ensenada, Baja California, Mexico
e-mail: tiagojp@cicese.mx

A. Rocha-Olivares
e-mail: arocha@cicese.mx

T. J. Pereira · M. Mundo-Ocampo
Department of Nematology, University of California, Riverside, CA 92521, USA

G. Fonseca
Centro de Biologia Marinha da Universidade de São Paulo, Rod. Manoel Hipólito do Rego km 131,5, São Sebastião 11600-000, Brazil

B. C. Guilherme
Centro de Educação e Saúde, CES, Universidade Federal de Campina Grande, Av. Olho D'Água da Bica s/n, Cuité, PB, Brazil

Introduction

Nematodes are the most dominant and diverse meiofaunal group in marine benthic habitats. Usually, they account for 70–90% of meiobenthic metazoan abundance in marine sediments, where they play fundamental ecological roles (Austen 2004). Estimates of marine nematode diversity are in the millions of species, of which only a small fraction has been described (De Ley et al. 2005; Lambshead and Boucher 2003). Unfortunately, the field of marine

nematology remains underdeveloped, perhaps due to labor-intensive techniques, a difficult taxonomy, and a very limited worldwide expertise (De Ley 2006; Bhadury et al. 2006).

Nematode systematics faces the fundamental question of whether morphological characters alone are sufficient to achieve a natural classification of all nematodes and whether these characters are reliable for species identification. The practical limitations and challenges imposed by natural variation in the identification of marine nematodes are many. For instance, taxonomic knowledge and identification keys are restricted to few geographic areas, and a high level of taxonomic expertise is needed to work with them. In addition to human-related limitations, natural variation in the form of phenotypic plasticity and cryptic/sibling speciation also imposes challenges making morphological variation taxonomically equivocal. This situation makes the use of new tools for nematode identification appealing and necessary.

Molecular approaches to describe, catalog, and identify biological diversity have been increasingly adopted in biodiversity studies, particularly since the inception of the DNA barcoding of life initiative (Hebert et al. 2003a, b; Blaxter 2004). Currently, these methods are being applied to a wide range of taxonomic groups (Floyd et al. 2002; Hebert et al. 2004; Ward et al. 2005; Meyer et al. 2008). The main goal of DNA barcoding is to characterize the biological diversity using a short DNA sequence that will facilitate and expedite taxonomic identification (http://www.barcoding.si.edu/). This initiative has helped in the discovery of new species, many of which have been shown to be morphologically cryptic, thereby considerably improving biodiversity assessment in poorly studied groups such as microscopic meiofauna (Rocha-Olivares et al. 2001; Blaxter et al. 2004; Derycke et al. 2005; Lessi and Todaro 2009). Among marine nematodes, the combination of molecular and morphological approaches has helped to disentangle different species complexes (e.g., Derycke et al. 2008; Fonseca et al. 2008).

DNA barcoding studies of marine nematodes have revealed a good degree of concordance between traditional morphology-based taxonomy and DNA sequences (De Ley et al. 2005; Bhadury et al. 2006, 2008). This is generally reflected in the correspondence between morphologically based Operational Taxonomic Units (OTU) and corresponding Molecular Operational Taxonomic Units (MOTU) (following Blaxter 2004 definitions). The advantages of molecular identification reside in its generally fast and easy implementation, as well as in the potential use of the molecular data for phylogenetic analyses. A partial sequence of the gene coding for the mitochondrial subunit I (*COX 1*) of the cytochrome c oxidase, originally proposed as the standard for DNA barcoding (Hebert et al. 2003b), has so far been unsuccessfully applied in marine nematodes mainly due to the unavailability of PCR primers working across the entire phylum (Blaxter et al. 2005; De Ley et al. 2005; Bhadury et al. 2006; Creer et al. 2010).

Even though no standardized gene for DNA barcoding is available for marine nematodes, an accumulation of nematode 18S or 28S nuclear ribosomal DNA (rDNA) sequences in public databases reflects their usefulness in molecular phylogenetic studies (Nadler 1992; Blaxter et al. 1998; Litvaitis et al. 2000; Nadler et al. 2006; Meldal et al. 2007). These data also reflect that the diverse order Enoplida Filipjev 1929 has been poorly explored compared to other marine nematode taxa by both molecular and classical taxonomy studies. Enoplids are present in nearly all marine sediments and represented by diverse species from several trophic levels (Platt and Warwick 1983). Some of them are predators and play an important role in regulating other nematode populations, mainly in intertidal sandy beaches where they are considered quite common (Greenslade and Nicholas 1991; Nicholas 2002).

The broad application of DNA barcoding to marine nematodes requires first finding a suitable genomic region, or combination of regions, for species identification across a variety of taxa. Second, it requires building a reference database of sequences and morphological vouchers from widespread localities, since most nematode sequences in molecular databases come from NW Europe. Preferably, the DNA barcoding region should have a "barcoding gap," which refers to a range of sequence divergence between species higher than and non-overlapping with the range of intraspecific divergence (Wiemers and Fiedler 2007). The aim of this study is to assess the marine nematode diversity of the order Enoplida from the coasts of Baja California using an integrative approach. Understanding the levels of concordance between morphological and molecular approaches through integrative taxonomy, as well as addressing the congruence of candidate gene regions for DNA barcoding (i.e., 18S and 28S genes), will help to improve our assessment of marine nematode diversity and their evolutionary relationships.

Materials and methods

Sampling, nematode extraction, and identification

Organisms were sampled in the intertidal zone of sandy beaches with a transparent corer (2 cm Ø x 10 cm) and were immediately fixed in the field with DESS solution (DMSO 20%, 0.25 M disodium EDTA, and saturated with NaCl, pH 8.0; Yoder et al. 2006). Four sites were sampled

in the Gulf of California: (1) San Felipe and (2) Santa Clara in the Upper Gulf of California (UGC), (3) Bahía de Los Ángeles close to the "Grandes Islas" region and (4) La Paz Bay at the southwestern end of the Gulf. On the Pacific coast, samples were collected from: (5) San Carlos and (6) Faro beach within Vizcaíno Bay and from (7) Cerritos beach at the southern end of the peninsula (Table 1).

Sediment samples were rinsed with tap water on a 63-μm sieve and the meiofaunal community was extracted by flotation with Ludox™ (specific gravity 1.15; Jenge and Steyaert 1977; Somerfield and Warwick 1996). Enoplids were individually picked out with a special brush under a dissection microscope (SZX7 OLYMPUS, 56X) and placed on a slide in a drop of sterile water. These temporary slides were analyzed under a compound microscope (OLYMPUS-BX51) with differential interference contrast. Anatomical details of each specimen were photographed at different magnifications to allow subsequent identification and measurements. Morphological identification of the specimens was based on the latest available keys for Enoplida (Sinel and Coomans 2006) and with the help of the database NEMYS (http://nemys.ugent.be/). Morphological vouchers for the specimens (digital microphotographs) are available on-line at the Nematode Tree of Life (NemATOL) web site (http://nematol.unh.edu/). After microscopic observation, which was performed as fast as possible to avoid DNA degradation, nematodes were subject e DNA extraction and PCR.

DNA extraction, amplification, and sequencing

Prior to DNA extraction, each specimen was rinsed (3 x) with sterile water to remove traces of DESS. Organisms were then transferred e a sterile slide containing 20 μl of Worm Lysis Buffer (WLB) (50 mM KCl, 10 mM Tris–Cl pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP40, and 0.45% Tween 20 as described in Williams et al. 1992) and 2 μl of proteinase K (10 mg ml^{-1} stock). Subsequently, organisms were cut into three or more pieces (depending on size) with asterile scalpel, transferred into 200-μl tube and frozen for 10–30 min at −20°C. Samples were incubated for 1 h at 65°C for protein digestion followed by 10 min at 95°C for proteinase inactivation. Finally, tubes were centrifuged for 1 min at 13,000 rpm. PCR amplifications were performed using 2.5 μl of the extraction supernatant.

Two rDNA genes were partially amplified by PCR: a fragment ca. 350 bp of the 18S gene (small subunit or SSU) and ca. 800 bp spanning the D2–D3 domains of the 28S gene (large subunit or LSU). The SSU gene was amplified using primers MN18F and a degenerate version of 22R (d22R) (GCCTGCTGCCTTCCTTRGA) from Bhadury et al. (2006). The D2D3 region was amplified

Table 1 Sampling sites

Region	Site[a]	Temperature (°C)	Salinity (‰)	Position (lat/long.)	Date	Habitat type
Gulf of California	1. San Felipe (SF)	32	35.5	31°1'34.58" /114°52'48.84"	07/2707	Pristine, reflexive beach, wide tidal range, gravel sand, small waves
	2. Santa Clara (SC)	30.5	35	31°4'17.98" /114°50'31.74"	07/2807	Pristine, dissipative beach, wide tidal range, fine sand (308 μm), small waves
	3. Bahía de Los Ángeles (BLA)	NA	NA	28°58'9.20" /113°32'27.03"	08/1508	Pristine, bay, wide tidal range, fine sand, no wave action
	4. La Paz (LP)	22	36	24° 9'55.62" /110°18'51.03"	11/1707	Disturbed, bay, fine sand, no wave action
Pacific coast	5. San Carlos (SCA)	NA	NA	25°57'21.72" /113°29'1.62"	05/2507	Little disturbed, dissipative beach, fine sand, can be affected by big waves
	6. Playa del Faro (PF)	26	36	24°14'21.58" /114° 6'13.62"	05/2407	Pristine, dissipative beach, medium sand, no wave action
	7. Cerritos (CE)	28	35	23°19'44.40" /110°10'32.88"	11/2108	Little disturbed, dissipative beach, fine-medium sand, can be affected by big waves

NA missing data

[a] Abbreviations in parentheses

using primers D2A and D3B from De Ley et al. (2005). Each 25 µl PCR reaction for SSU consisted of 2.5 µl DNA, 10 µl dNTPs (0.5 mM each), 2.5 µl 10× PCR buffer (1.5 mM $MgCl_2$), 1 µl of each primer (10 µM), 1 U of *Taq* polymerase (New England Biolabs, 5 U/µl) and ddH_2O. The low amplification success of the LSU gene using *Taq* was partially solved using a high-performance DNA polymerase with the following protocol: 1.5 µl dNTPs (0.5 mM for each), and 0.75 µl of DyNAzyme™ EXT polymerase (Finnzyme, 1 U/µl).

After verification in a 1.5% agarose gel stained with ethidium bromide (0.5 µg ml^{-1}), PCR products were purified for sequencing using exonuclease and shrimp alkaline phosphatase digestion with EXOSAP-IT (USB Affymetrix, Inc.) following the manufacturer's protocol. Both rDNA genes were sequenced in both directions with PCR primers using ABI-PRISM® Dye-DeoxyTerminator Big Dye™ v3.1 (Applied Biosystems Inc, CA) with an automatic sequencer Gene Analyzer ABI 3100 (Applied Biosystems Inc, CA).

Data analysis

Morphometric analyses included standard characters used in nematode systematics such as body length (*L*), body width (*W*), pharynx length (*Ph*), nerve ring position (*nr*), buccal cavity length (bc L), buccal cavity width (bc W), head width (h W), anterior cephalic setae length (acs L), posterior cephalic setae length (pcs L), anal body diameter (abd), tail length (Tail), and shape parameters *a* (*L*/*W*), *b* (*L*/*Ph*), and *c* (*L*/Tail). For most taxa with few specimens or with low phenotypic variability, morphometric data (mean and range) were compared with those in the literature for identification. For the three closely related species of *Metacanthion* (*M.* sp1, *M.* sp2 and *M.* sp3), statistical analyses were carried out to establish the significance of their morphological distinction, which suggested the existence of several species. For these specimens measurements were used to compute pair-wise Euclidean distances among individuals. Non-metric multidimensional scaling (MDS) was used to assess morphological differentiation on a low dimensional space. Significant differentiation ($P < 0.05$) among groups was assessed with analysis of similarity (ANOSIM, Clarke and Gorley 2001). Finally, we used analysis of similar percentages (SIMPER) to identify which morphological characters contributed most to the differentiation among groups. Sequential Bonferroni correction was applied to significance levels to adjust for non-independent multiple comparisons (Rice 1989).

DNA sequences were edited with CodonCode Aligner 2.0.1 and subsequently aligned in ClustalX with default parameters (Thompson et al. 1997). Phylogenetic relationships among sequences were estimated with maximum parsimony (MP), maximum likelihood (ML) and neighbor joining (NJ), using heuristic searches in PAUP 4.0b (Swofford 1998). For ML searches, we used the AIC criterion to find the best-fit model of molecular evolution (18S: TVMef+I+G and 28S: GTR+I+G) and its parameters with the programs Modeltest 3.7 (Posada and Buckley 2004; Posada and Crandall 1998) and PAUP 4.0b (Swofford 1998). NJ searches were performed on ML distances computed with the best-fit model of molecular evolution. Non-parametric bootstrap was used to assess branch support (MP and NJ: 1,000 pseudoreplicates; ML: 100 pseudoreplicates).

The incongruence length difference test (ILD test; Farris et al. 1994) was used to determine if tree topologies obtained from both rDNA genes were significantly incongruent. This analysis was carried out using MP heuristic searches and 1,000 bootstrap pseudoreplicates in PAUP 4.0b. Finally, phylogenetic trees were inferred from both genes under MP and ML. For ML, the model of molecular evolution was readjusted for both genes (GTR+I+G) following the AIC criterion in Modeltest 3.7 (Posada and Crandall 1998; Posada and Buckley 2004).

In discovering MOTUs, we adopted a cut-off level of 99.5% sequence similarity (equivalent to no more than 0.5% sequence divergence) among specimens to be assigned to the same MOTU.

Results

Morphological identification

We isolated 139 enoplid specimens from the 7 coastal localities and recognized 22 possible morphological species. From these, 20 were identified to generic level (14 genera, 6 families), one specimen was determined only to family level (Thoracostomopsidae), and another could not be identified beyond the order Enoplida (Table 2). Thoracostomopsidae and Oncholaimidae were the most abundant families with seven and three genera, respectively; Enchelididae included two genera, and the rest of the families (Ironidae, Tripyloididae, and Rhabdodemaniidae) only one.

Even though organisms identified in this study showed qualitative similarities with species already described in the literature (*Trileptium* sp2 similar to *T. parisiense*, *Oxyonchus* to *O. dentatus* and *O. avalysae*, *Enoploides* to *E. brunetti* and *E. longipiculatus*, *Epacanthion* to *E. cavani* and *E. alifil*), morphometric characters suggest that many of our species are new to science (Table 3). In addition, the large unidentified Thoracostomopsidae from Santa Cruz (>5 mm in length) differed from all known genera reported in the literature, and was mainly distinguishable by its hemispherical tail, making it a candidate for a novel genus.

Table 2 Taxonomic list of identified families and genera, number of individuals processed for each morphological species and number of sequences produced for both genes

Order	Family	Genus	Site	Num. (ind.)	Num. (Seq.)	
					18S	28S
Bryplida	Brachildidae	*Cyclopronema*	LF	1	0	0
		Neoxystomina	IC	2	0	2
	Ironidae	*Pleurotholis*	SLA	1	0	0
	Onchulembidae	*Metoncholaimus*	IF	8	6	2
		Oncholaimus	LF	9	2	2
		Viscosia	SCA	8	2	2
	Rhabdolaimatidae	*Rhabdolaimoides*	IC	9	6	2
	Thoracostomopsidae	*Bicoplides*	IF	20	9	10
		Epacanthion	IC	29	9	3
		Mesacanthion sp1	SLA	10	2	2
		Mesacanthion sp2	SF	15	6	6
		Mesacanthion sp3	CIL	11	4	4
		Mesacanthion sp4	FF	1	1	1
		Mesacanthion sp5	SCA	1	1	1
		Mesacanthoides	IC	2	2	1
		Oxystomina	IC	2	2	1
		Thoracostomopsid	SC	2	2	2
		Thalpirion sp1	SC	2	2	1
		Thalpirion sp2	SC	3	3	3
		Thalpirion sp3	FF	1	1	1
	Tripyloididae	*Bathylaimus*	IC	1	1	1
	Bryplid	Bryplid	SCA	1	1	1
Total				139	62	48

Site abbreviations are presented in Table 1

Nematodes of the genus *Mesacanthion* were sorted into five species, based on morphology and molecular data, two in the Gulf of California (*M.* sp1 and *M.* sp2) and three in the Pacific coast (*M.* sp3, *M.* sp4, and *M.* sp5). *Mesacanthion* sp1, *M.* sp2, and *M.* sp3 were morphologically similar having a conic-cylindrical tail ($n = 8$, 6, and 5, respectively). An MDS plot showed a clear segregation of organisms into three morpho-groups suggesting distinct morphological species (Fig. 1). ANOSIM confirmed significant differentiation among them (*M.* sp1 × *M.* sp2, $P = 0.001$ and $R = 0.652$; *M.* sp1 × *M.* sp3, $P = 0.001$ and $R = 0.992$; *M.* sp2 × *M.* sp3, $P = 0.002$; $R = 0.768$. P values significant after sequential Bonferroni correction). Four morphological characters were mainly responsible for this differentiation as revealed by SIMPER. Differences in body ratios *a* (body length/width), *b* (body length/pharynx length), and *c* (body length/tail length) as well as differences in *L* (body length) accounted for 70–80% of the cumulative differentiation among *M.* sp1, *M.* sp2, and *M.* sp3.

Mesacanthion sp1, sp2, and sp3 were morphologically similar to *M. alexandrinus* in having somatic setae along the body (mainly in the anterior region), a long and slender spicule with a small gubernaculum and one small pocket shape supplement among males. On the other hand, morphometric differences (average and range values) were observed among these species for characters such as body length (*M.* sp3 < *M.* sp1 < *M. alexandrinus* < *M.* sp2), pharynx length (*M.* sp1 < *M.* sp2 < *M.* sp3 < *M. alexandrinus*), nerve ring position (*M.* sp1 < *M.* sp3 < *M.* sp2 < *M. alexandrinus*), and tail length (*M. alexandrinus* < *M.* sp3 < *M.* sp2 < *M.* sp1, Fig. 2). Based on these comparisons, *M.* sp2 seems to be more similar to *M. alexandrinus* than *M.* sp1; however, they still differ in vulva position (Table 3).

18S and 28S sequences

The DNA of 110 from the 139 analyzed nematodes could be successfully amplified and sequenced (18S = 62 and 28S = 48, Table 2). We identified 19 distinct 18S and 20 28S (D2D3 region) MOTUs. Sequences are available on GenBank under accession numbers: 18S, GU130747–GU130765; 28S, GU130766–GU130785. PCR success was different between genes, and was considerably higher (137/139 or 99%) with the short 18S fragment than with

Table 3 Morphometric comparison (mean and range in μm) between some of the morphological species identified in this study and species already described in the literature

Species	Sex	L	W	Ph	nr	a	b	c	Tail	V%
Epacanthion sp. from SC (n. sp.), 4 males, 4 females	M	3440 (3330–3700)	70 (70–80)	930 (88–1040)	140	46.0 (44.9–47.5)	3.7 (3.6–3.8)	15.1 (12.2–15.9)	260 (260–270)	
	F	3670 (3390–3780)	100 (90–110)	990 (970–1030)	150	37.0 (36.8–37.2)	3.6 (3.5–3.7)	14.4 (13.0–15.8)	250 (240–260)	58.6 (58.0–59.2)
Epacanthion [illegible] Keppner, 1986, 3 males, 2 females	M	2110 (2050–2170)	51 (50–53)	538 (525–564)	122 (117–128)	41.4 (39.8–43.4)	3.9 (3.9–4.0)	16.5 (16.0–16.9)	128 (127–130)	
	F	2580 (2530–2690)	62 (61–63)	595 (589–598)	131 (128–135)	41.6 (41.5–41.7)	4.3 (4.2–4.4)	15.7 (15.7–15.8)	164 (160–167)	59.0
Epacanthion [illegible] Inglis, 1966, 2 males, 3 females	M	1995 (1880–2110)	54.5 (54–55)	615 (590–640)	135.5 (131–140)	36.6 (34.8–38.4)	3.1 (3.2–3.1)	10.2 (10.1–10.3)	195 (182–208)	
	F	1913 (1850–2080)	64.3 (58–73)	616.7 (530–680)	155 (138–168)	30 (25.3–32.6)	3.2 (2.9–3.5)	10.9 (9.0–12.1)	182 (138–165)	59.7 (58.4–61.7)
Enoploides sp. from SF, 2 males, 4 females	M	2530 (2170–2490)	70 (70–70)	690 (640–730)	140	35.3 (33.7–36.8)	3.4 (3.4–3.4)	18.6 (17.7–19.6)	130 (120–130)	
	F	3170 (2230–3920)	80 (70–80)	700 (620–740)	150 (130–160)	32.5 (31.1–34.9)	3.52 (3.4–3.6)	17.4 (15.7–18.7)	140 (140–150)	65.4 (63.8–66.4)
Enoploides brunettii Gerlach, 1953, 2 males, 1 female	M	2120 (2060–2181)	65.5 (57–74)	460 (450–470)	?	32.5 (29–36)	4.55 (4.5–4.6)	15.35 (14.7–16)	141 (135–147)	
	F	2128 (2500–3000)	67 (69–75)	435	?	32	4.7	16	130	59.0 (58.0–59.0)
Enoploides longispiculosus Vitiello, 1967	F	(3100–3900)	(92–115)	?	?	(35–38)	?	?	?	(56–58)
Mesacanthion sp1 from BLA (n. sp.), 4 males, 4 females	M	2000 (1890–2180)	50 (50–60)	420 (380–470)	110 (100–120)	36.6 (33.5–38.6)	4.8 (4.4–5.3)	13.1 (12.4–14.3)	150 (140–170)	
	F	1980 (1900–2113)	50 (50–60)	410 (380–440)	110 (100–120)	35.8 (32.3–42.7)	4.8 (4.7–5.0)	12.9 (12.4–13.4)	150 (130–160)	58.3 (57.7–59.0)
Mesacanthion sp2 from SF (n. sp.), 1 male, 1 female, 4 juveniles	M	2870	60	510	130	49.5	5.6	19.0	150	
	F	3170	60	440	130	40.6	5.3	18.3	140	52.3
	J	2240 (1970–2540)	50 (50–60)	440 (410–480)	120 (110–130)	44.5 (42.8–46.2)	5.1 (4.7–5.7)	16.5 (14.0–17.9)	140 (130–140)	
Mesacanthion sp3 from CE (n. sp.), 4 female	F	1870 (1700–1940)	50	510 (470–550)	110 (110–120)	38.3 (33.9–41.6)	3.6 (3.5–3.9)	14.33 (13.8–15.4)	130 (120–140)	61.4 (60.4–62.0)
Mesacanthion [illegible] Nicholas, 1993, 4 males, 3 females	M	1830 (1150–2460)	47 (37–59)	509 (430–578)	134 (114–158)	39.2 (33–43)	3.4 (3.1–3.6)	16.5 (14–2)	111.5 (97–110)	
	F	2167 (2270–2570)	71 (60–86)	681 (594–730)	164 (151–180)	35.7 (26–42)	3.6 (3.5–3.8)	18 (17–19)	137.3 (136–139)	57.7 (55–63)
Oxyonchus sp. from SC, 2 males	M	2560 (2210–2910)	80	750 (720–780)	135 (130–140)	31.9 (28.3–35.5)	3.4 (3.1–3.8)	9.7 (8.8–10.5)	260 (250–280)	
Oxyonchus [illegible] Nicholas, 2004, 4 males	M	2134 (1964–2307)	76.83 (70.43–81.09)	599 (550–634)	129 (119–146)	46 (38–53)	3.6 (3.3–3.8)	10 (9–12)	225 (196–252)	
Oxyonchus dentatus Filipjev, 1927, 1 male	M	4500	100	768	?	45	5.9	15	300	
Trileptium sp2 from SC (n. sp.), 1 male, 1 female	M	3310	50	1100	320	66.2	3.0	18.4	180	
	F	3500	50	640	160	70	5.5	17.5	200	61.3
Trileptium parisetum Warwick and Platt, 1973, 1 male	M	3800	41	722	?	92.7	5.3	23.1	164.7	

Morphometric characters and sites abbreviations were presented in Materials and methods and Table 1, respectively. Data were taken from original descriptions and from Platt and Warwick ([illegible], in bold). ? denotes missing data

SC Santa Clara, *SF* San Felipe, *BLA* Bahía de Los Ángeles, *CE* Cerritos

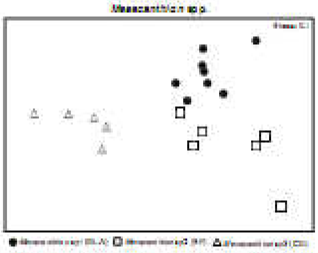

Fig. 1 Non-metric multidimensional scaling (MDS) plot obtained from the morphometric data of *Mesacanthion* spp. with conico-cylindrical tails. Site abbreviations are in Table 1

the D2D3 region (93/139 or 67%). On the other hand, sequencing success rate was very similar between genes and only a few PCR products could not be successfully sequenced. This was the case of *Paraeurystomina* for the 18S gene and *Trissonchulus* and *Calyptronema* for both genes. The alignment of the 18S gene was 324 bp long, including gaps, and the fraction of variable sites was smaller than the fraction of conserved sites (Table 4). On the other hand, the 28S gene alignment was 794 bp long, contained more gaps, and was more polymorphic than the SSU alignment. In this gene, the percentage of variable sites more than doubled the percent of conserved sites (Table 4).

Comparison of our sequences with those in GenBank revealed high similarities with confamilial and congeneric sequences, and in some cases with sequences from specific taxa. Sometimes 18S and 28S sequences from the same specimen provided matches to different taxa, which likely resulted from the absence of more closely related species in the database (see electronic supplementary material). For instance, *Viscosia* specimens sampled in San Carlos were 99% similar to the 18S from *Viscosia viscosia* (DQ394740) with 100% of coverage; 95 and 91% similar to the 28S sequence from *Oncholaimus* sp. (AF210413) and *Viscosia* sp. (DQ077751) with 43 and 100% of coverage, respectively.

Morphological and molecular approaches were concordant in species identification. Each morphological species with replicate specimens possessed a unique rDNA

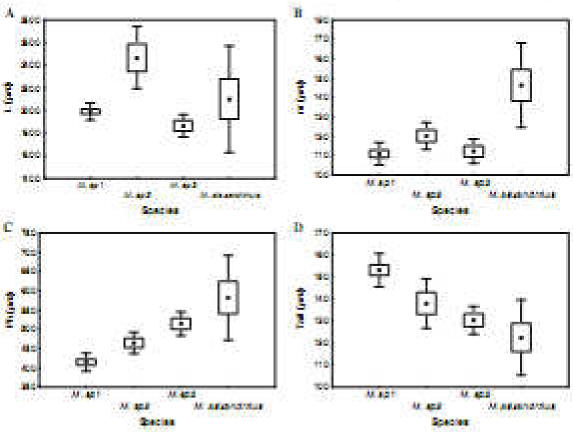

Fig. 2 Whisker plots of mean, standard deviation and range of morphometric variables among three closely related *Mesacanthion* spp. identified in this study and *M. alexandrinus* (Nicholas 1993). a Body length, b nerve ring position, c pharynx length, and d tail length

Table 4 Variability and composition of the 18S and 28S rDNA sequences

Gene	Alignment size (bp)	Variable (bp)*	Conserved (bp)	Parsimony informative (bp)*	Nucleotide frequencies (%)			
					T(U)	C	A	G
18S	824	158 42.6%	183 56.5%	111 34.26%	27.0	21.5	27.9	23.6
28S (D2D3 region)	794	545 68.6%	240 30.2%	457 57.56%	24.2	21.4	25.4	29.0

Alignment including gaps, number of variable, conserved sites and parsimony informative sites (bp and percentage) and nucleotide frequencies (percentage)

* Excluding indels

* Indels were coded as missing data in MP analyses

sequence in both genes resulting in a single MOTU. Although not all the specimens were sequenced, most of the species had two sequences for intraspecific comparisons, at least for one of the genes (Table 2). MOTU divergences increased with decreasing relatedness in both genes; however, divergence was consistently higher for the 28S rDNA (Fig. 3).

We found morphological variation among specimens of *Enoplolabs* and *Meracanthion* sp3 potentially interpretable as the presence of more than one species. In *Enoplolabs*, a male showed features (head shape, jaws, and body size) very different from the rest of congeneric males and females. *Meracanthion* sp3 specimens, on the other hand, were entirely females and juveniles with very different morphological features (mainly body shape and head, electronic supplementary material); nevertheless, they shared identical DNA sequences of both genes. The same was true for the specimens of *Enoplolabs*. Consequently, this morphological variation was not interpreted as indicative of additional taxa.

Phylogenetic analyses

The three methods of phylogenetic reconstruction (ML, MP, and NJ) produced highly congruent 18S gene trees, featuring three major clades (Fig. 4a). A highly supported clade (bootstrap ≥ 90%) consisted of all the Thaumastoxenopsidae sequences; a second grouped *Bathylabrus* and (non-identified) Enoplida n.i. (bootstrap 100%); a third moderately supported clade grouped the three onchelaimid genera (*Viscosia*, *Metoncholaimus*, and *Oncholaimus*) with *Rhabdolaimoides* (Rhabdolaimatidae) (Fig. 4a). Thaumastoxenopsidae and Oncholaimidae were monophyletic in all trees, and the latter received the highest bootstrap support in all reconstructions. Whereas the three oncholaimid genera were completely resolved, some relationships among the more numerous Thaumastoxenopsid genera were not. The five sequences of *Meracanthion* spp. were not monophyletic. Whereas the closely related species *M.* sp1 and *M.* sp2 were grouped with a maximum support, *Epacanthion* and *M.* sp3 were joined in the same clade with modest support values (Fig. 4a).

On the other hand, 28S gene trees were less consistent. The main difference among the inference methods was the position of the enchelidiid genus *Hieronymiella* (Fig. 4b, in bold). In the MP reconstruction, this genus was sister to Oncholaimidae and relationships were completely resolved with families reciprocally monophyletic. The relationship of *Viscosia* as sister to *Oncholaimus* and *Metoncholaimus* was moderately supported (60% bootstrap). In the ML and NJ trees, Oncholaimidae and Enchelidiidae were paraphyletic, although relationships within these families were

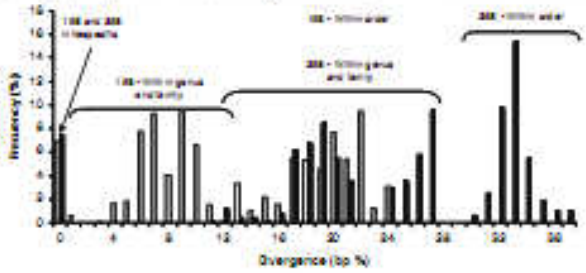

Fig. 3 Frequency distribution of pairwise sequence divergence in Enoplida for 18S (*white bars*) and 28S (*black bars*) rDNA. Number of pair-wise comparisons: 18S: 1770, 28S: 1035

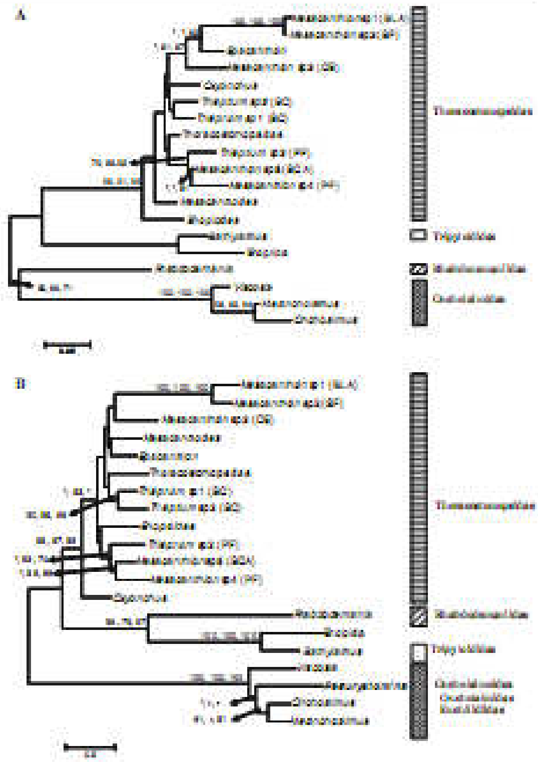

Fig. 4 Maximum likelihood phylogenetic reconstructions of Enoplida sequences from Baja California. **a** 18S gene, **b** 28S gene. Bootstrap values (only >50%) for the different methods (MP, ML and NJ, respectively) are shown on branches (– bootstrap <50% or no value at all, * branch absent). Families are indicated by *vertical bars*

poorly resolved. However, the taxa comprising both families grouped in a monophyletic Oncholaimoidea (Fig. 4b).

Since only one Enchilidiid was present in our samples, an additional sequence was obtained from GenBank (28S: *Calyptronema maxweberi*, AF210399) to address relationships between Enchilidiidae and Oncholaimidae. The reciprocal monophyly of the families was not supported by our data: MP produced a polytomy joining both enchilidiids (*Pareurystomina* and *C. maxweberi*) with the monophyletic oncholaimid sequences; ML produced a paraphyletic Enchilidiid pair (Fig. 5b). The monophyly of the superfamily Oncholaimoidea (Enchilidiidae + Oncholaimidae) in these analyses was consistently and strongly supported (Fig. 5).

The phylogenetic signal contained in each rDNA gene produced topological differences in the phylogenies. For instance, the genus *Rhabdodemania* was sister to Enoplida n.i. + *Bathylaimus* in the 28S tree but sister to all Oncholaimidae in the 18S reconstruction; both nodes were well supported by at least two of the three methods (MP, ML, NJ). Other differences were found among Thoracostomopsid genera, particularly in the deeper nodes leading to

Fig. 5 a Maximum parsimony and b maximum likelihood phylogenetic reconstructions of Enoplida 28S rDNA sequences from Baja California including *Calyptronema maxweberi* sequence from GenBank (AF210399.1). Bootstrap values (only >50%) are shown on branches

their common ancestor. Based on the number of nodes with high support values, both genes appear to have comparable resolving power but for different taxa.

In spite of these topological differences, the 18S and 28S trees were congruent (ILD test, $P = 0.28$), suggesting that differences were the result of poorly resolved relationships in both data sets. Therefore, we proceeded to merge the sequence data for a combined analysis (1,123 characters, including gaps). MP and ML (evolution model readjusted to GTR+G+I) produced very similar trees with three very well-supported main clades: (1) Thoracostomopsidae, (2) Oncholaimidae and (3) *Rhabdodemania*, *Rathylaimus* and Enoplida n.i. (Fig. 6); of these only the latter received less than 90% bootstrap support in the ML reconstruction (Fig. 6b). Within Thoracostomopsidae, ML produced a larger number of better supported clades, including the monophyly of the three closely related *Metacanthion* spp., which were paraphyletic in the MP consensus tree (Fig. 6a). *Trillopthus* sequences were consistently paraphyletic in all phylogenetic reconstructions. Only the monophyly of sympatric *Trillopthus* sp1 and sp2 was recovered with high support values in all analyses, whereas *Trillopthus* sp3 from Playa del Faro was paraphyletic with *Metacanthion* sp4 and *M.* sp5 in both trees (Fig. 6).

Discussion

The marine nematode communities of the Gulf of California are poorly known and data are available from only two localities in the EGC (Mundo-Ocampo et al. 2007; Holovachov et al. 2008), and there is no information available from the Pacific coast of Baja California. Thus, this study focused on enoplids reveals novel insights about the distribution of marine nematodes in other areas of the Gulf of California as well as the first data from the Pacific coast of Baja California, Mexico.

Integration of morphological and molecular approaches for identification

Morphological identification of the enoplids in this study was supported by the molecular data, as shown by the congruence and high similarity between our sequences and those available in GenBank. Discrepancies between our morphological identifications and the closest matches in the molecular database are most likely the result of the limited taxonomic coverage of rDNA sequences available in GenBank relative to the vast diversity of marine nematodes (Lambshead and Boucher 2003). The limited sequence availability and the unavailability of morphological cross-referenced vouchers in GenBank preclude using these Blast analyses as a bona fide molecular identification tool. Pending the sampling of additional molecular data of closely related taxa, they are nevertheless consistent with our morphological determinations.

In addition, molecular data also revealed that some conspicuous morphological variation between conspecific specimens could be ontogenetic (*M.* sp3, differences between juvenile and adult) or due to phenotypic plasticity (*Enoploides*, differences between adults). Ontogenetic variation is an important issue in nematode identifications, since most diagnostic characters relate to adult (often male) genitalia absent in subadults, which may be the only life-stage sampled (De Ley 2006; Bradury et al. 2006). On the other hand, marine nematodes seem to be phenotypically

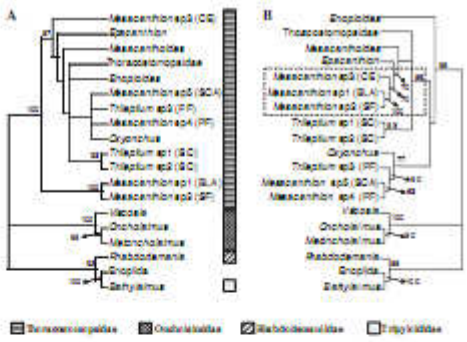

Fig. 6 Phylogenetic reconstruction using 1,123 bp from 18S and 28S rDNA genes combined of Enoplida nematode from Baja California, a MP consensus tree of five most parsimonious reconstructions. b ML tree based on GTR+G+I model of sequence evolution. Bootstrap values (only >50%) are shown on branches

plastic, which is a source of taxonomic uncertainty and may mislead and bias diversity studies based exclusively on morphology (Nadler 2002; Derycke et al. 2008). Additional molecular data from more polymorphic genes would be required to test the hypothesis that rDNA sequences were insufficiently variable to reflect interspecific divergence if the observed phenotypic variation relates to differences between species instead of intraspecific plasticity.

Surprisingly, two of the *Meacanthion* spp. (*M.* sp1 and *M.* sp2) found in the Gulf of California presented morphological features very similar to *M. alexandrinus* described by Nicholas (1993) from a freshwater environment in Australia. Despite their qualitative resemblance, the contrasting habitats where they were found (freshwater vs. marine) and the levels of phenotypic differentiation suggest that *Meacanthion* spp. from this study may represent species new to science. Based on the integration of morphological and molecular data, we also showed that the three closely related *Meacanthion* spp. (*M.* sp1, *M.* sp2 and *M.* sp3) are different from each other and therefore should be treated as different species. The unidentified Thoracostomopsidae differed from all genera known and reported in the literature. We could find no resemblance to any genus described for the entire order Enoplida, even to those described from freshwater environments (Smol and Coomans 2006), suggesting that this nematode may represent a new genus of Thoracostomopsidae. A detailed morphological description of these specimens is beyond the scope of this paper and will be presented elsewhere.

Morphological and molecular approaches were congruent in addressing nematode species identification since (1) each species identified based on morphology presented a unique DNA sequence (MOTU), and (2) sequences were phylogenetically concordant with taxonomy, for most part. Moreover, the combination of both approaches showed that natural variability (ontogenetic development, sibling and cryptic species) could bias biodiversity evaluation, over- or underestimating the number of species. An integrative taxonomic approach is the best strategy for marine nematode identification as previously suggested (Coomans 2002; De Ley 2006; Bhadury et al. 2006; Derycke et al. 2008).

Relative merit of 18S and 28S genes for DNA barcoding

Both genes, 18S and 28S, produced an equal number of unique sequences (MOTU diversity) showing the same capability of addressing nematode species identification. The smaller 18S fragment showed a considerably higher amplification success; however, the lower polymorphism may limit its phylogenetic usefulness. On the other hand, divergence was much higher in the D2D3 domain of the 28S gene, regardless of taxonomic level (i.e. families or genera), making it suitable for both DNA barcoding and phylogenetic reconstruction of marine nematodes (De Ley et al. 2005; Derycke et al. 2008). This increased divergence would be particularly valuable in resolving closely related species, as in the case of *Meacanthion* (*M.* sp1 and *M.* sp2, 18S = 1.06% and 28S = 12.09%) and *Tripylium* (*T.* sp1 and *T.* sp2, 18S = 3.87% and 28S = 12.97%), and to

detect possible cryptic species, where morphological differences are sometimes impossible to diagnose (De Ley et al. 1999; Fonseca et al. 2008). However, 28S had limited success in PCR amplifications (67%). A gene used for DNA barcoding should be easily reproducible across the entire Nematoda phylum. The value of the D2D3 region as a potential barcoding gene has been shown in studies of plant parasitic and free-living nematodes (De Ley et al. 1999; Subbotin et al. 2005; Subbotin et al. 2007). De Ley et al. (2005) have shown a high success rate in PCR amplification between different groups of nematodes; consequently, increased success for marine nematodes may require additional PCR optimization or the design of new nested primers encompassing diagnostic regions (Fonseca et al. 2008).

Phylogeny

In general, phylogenetic inferences from both genes were not intrinsically different among methods (MP, ML and NJ). In fact, tree topologies for the 18S gene were completely congruent. Differences among 28S reconstructions involved only the position of the Enchelidiid *Hemerycystoides* (Fig. 4e). Inclusion of an additional Enchelidiid sequence (28S: *Calyptronema maxweberi*, AF210999.1) did not help resolving the reciprocal monophyly of Enchelidiidae (*Hemerycystoides* and *C. maxweberi*) and Onchholaimidae (*Viscosia*, *Onchholaimus*, and *Metacholaimus*) (Fig. 4). Additional taxa from both families may be needed to resolve this node, should the lack of resolution be a result of insufficient taxon sampling.

This study also revealed contrasting phylogenetic signals in 18S and 28S genes. Although the ILD test did not detect significant differences between tree topologies ($P = 0.28$), the genus *Rhabdolaimoides* showed a controversial position in the 18S reconstruction. In morphology-based and other molecular phylogenies (e.g., Litvinova et al. 2010), *Rhabdolaimoides* (Rhabdodemanidae) and *Bathylaimus* (Tripyloididae) are grouped in the suborder Tripyloidina, in concert with the topology obtained with the D2D3 fragment (Fig. 4).

Our results underline the need for a combination of morphological and molecular approaches to expedite our understanding about marine nematode taxonomy, biogeography, dispersal capacity and gene flow among populations. These approaches will prove invaluable to have a fresh understanding of real levels of cosmopolitanism in marine nematodes, as it has been extensively reported in the literature (Bhadury et al. 2008; Derycke et al. 2005; Fonseca et al. 2006; Heip et al. 1985). Finally, an integrative approach will aid in the detection of cryptic species, which are common among meiofaunal groups, thereby improving the assessment of marine nematode diversity and contributing to a more robust nematode taxonomy (Derycke et al. 2007; Rocha-Olivares et al. 2001; Tchesunov et al. 1996).

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