Universidade Federal de Minas Gerais

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Departamento de Zoologia

Pós-Graduação em Zoologia

FILOGENIA MORFOLÓGICA DA FAMÍLIA XENOTRICHULIDAE (GASTROTRICHA)

THIAGO QUINTÃO ARAÚJO

Belo Horizonte - MG

Maio/2017

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FILOGENIA MORFOLÓGICA DA FAMÍLIA XENOTRICHULIDAE (GASTROTRICHA)

Tese apresentada como requisito parcial à obtenção do grau de Doutor em Zoologia. Programa de Pós-Graduação em Zoologia, Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais.

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Coorientador: Dr. André Rinaldo Senna Garraffoni

Belo Horizonte

Maio/2017

The food might be bad, and the beds might be hard. But at least we're living our own lives. And nobody else's.

Supernatural, Scarecrow episode.

Be ashamed to die until you have won some victory for humanity.

Horace Mann

AGRADECIMENTOS

Há 9 anos eu começava a trabalhar com esses maravilhosos animais que vivem entre os grãos de sedimento dos ambientes aquáticos. Não só foi um prazer enorme estudá-los, como é incrível perceber como a minha vida foi afetada por esses minuscúlos animais. Conheci países, cidades, Estados, pessoas, pesquisadores, os quais marcaram profundamente minha vida, e à todos devo eterna gratidão.

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Aviso

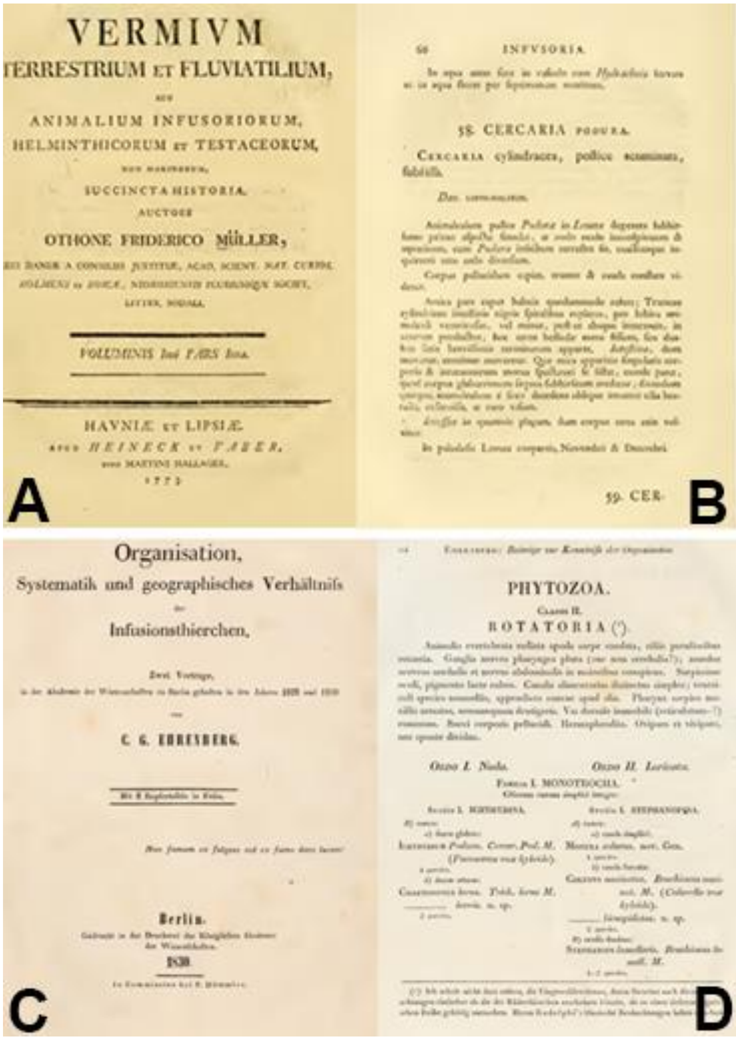
Este trabalho é parte integrante dos requerimentos necessários à obtenção do título de doutor em Zoologia, e como tal, não deve ser vista como uma publicação no senso do Código Internacional de Nomenclatura Zoológica (artigo 9) (apesar de disponível publicamente sem restrições) e, portanto, quaisquer atos nomenclaturais nela contidos tornam-se sem efeito para os princípios de prioridade e homonímia. Desta forma, quaisquer informações inéditas, opiniões e hipóteses, bem como nomes novos, não estão disponíveis na literatura zoológica. Pessoas interessadas devem estar cientes de que referências públicas ao conteúdo deste estudo, na sua presente forma, somente devem ser feitas com aprovação prévia do autor.

Notice

This work is a partial requirement for the PhD degree in Zoology and, as such, should not be considered as a publication in the sense of the International Code of Zoological Nomenclature (article 9) (although it is available without restrictions) therefore, any nomenclatural acts herein proposed are considered void for the principles of priority and homonymy. Therefore, any new information, opinions, and hypotheses, as well as new names, are not available in the zoological literature. Interested people are advised that any public reference to this study, in its current form, should only be done after previous acceptance of the author.

Apresentação

Gastrotricha é um filo composto por microinvertebrados de vida livre, encontrados em ambientes marinho, estuarino ou de água doce amplamente distribuídos pelo mundo. A história taxonômica deste táxon teve início há mais de 250 anos atrás. Müller (1773) (Fig. 1A-B) descreveu duas espécies novas com o formato de "pino de boliche" coletados, *Trichoda larus*, que foi descrito como um ciliado e *Cercaria podura,* que, por sua vez foi descrito como Platyelminthes (Fig. 1B). Ehrenberg (1830) (Fig.1C-D) observando a morfologia dos animais descritos por Müller (1773), sugeriu que estas duas espécies pertenciam aos rotíferos de água doce, transferindo essas espécies para dois novos gêneros descritos neste trabalho: *Ichthydium* (*Ichthydium podura*) e *Chaetonotus* (*Chaetonotus larus*) (Fig. 1D).


VERMIVM
TERRESTRIUM ET FLUVIATILIUM,
ANIMALIUM INFUSORIORUM,
HELMINTHICORUM ET TESTACEORUM,
SUCCINCTA HISTORIA.
AUCTORE
OTHONE FRIDERICO MÜLLER,
HAVNIÆ ET LIPSIÆ.
APUD HEINECK ET FABER.
A

INFUSORIA
58. CERCARIA PODURA.
59. CER.
B

Organisation,
Systematik und geographisches Verhältniss
der
Infusionsthierchen.
C. G. EHRENBERG.
Berlin.
1830.
C

PHYTOZOA.
ROTATORIA (*).
D

Figura 1 - A – Capa do traballho de Muller 1773, B – Descrição da espécie *Cercaria podudura* . C - Capa do traballho de Eherenberg, 1830. D - Descrição dos gêneros *Chaetonotus and Ichthydium* e sinonimização das espécies descritas por Muller 1773.

Mecnikow (1865) notou pela primeira vez a ciliação ventral desses animais com o formato de pino de boliche e nomeou um táxon exclusivo para os portadores desta peculiar característica (Gastrotricha: Greek *gaster*: estomago, and *thrix*: pêlo). O primeiro estudo com objetivo de descrever a biologia, morfologia e propor uma classificação para os gastrótricos até então conhecidos foi proposto por Zelinka (1889). Contudo, esse último autor ainda manteve os gastrótricos como um subgrupo dos rotíferos. Quase meio século depois, Remane (1936) sintetizou em seu trabalho todo o conhecimento sobre a sistemática de Gastrotricha, retirando esse táxon de dentro dos rotíferos e classificando-o como um grupo a parte pertencente aos "Aschelmintes".

Atualmente, o Filo Gastrotricha possui cerca de 820 espécies formalmente descritas (Araújo et al. 2015; Balsamo et al. 2015; Kieneke et al. 2015; Kolicka et al. 2015, 2016; Garraffoni et al. 2016b; Kånneby 2016), e foi dividido classicamente em duas ordens, Macrodasyida and Chaetonotida (mas veja Kieneke et al. 2008). A ordem Chaetonotida é composta por cerca de 480 espécies que possuem o corpo com o formato de "pino de boliche", faringe trirradiada com formato em "Y" e com maior parte do grupo partenogenético. Espécies desse grupo são encontrados em todos os ambientes aquáticos, mas sua grande maioria, cerca de dois terços do grupo, se concentram em ambientes de água-doce. (Balsamo and Todaro 2002; Balsamo et al. 2013, 2015; Garraffoni et al. 2016a).

Pertencente a Chaetonotida, os Xenotrichulidae Remane, 1927, são gastrótricos marinhos encontrados em comunidades bentônicas das zonas de litorâneas e sublitorâneas. Antes do presente estudo, o grupo era composto por 25 espécies formalmente descritas em três gêneros, sendo 15 espécies pertencentes ao gênero *Xenotrichula*, nove espécies pertencentes ao gênero *Heteroxenotrichula* e uma espécie do gênero *Draculiciteria.*

Até o momento os estudos relacionado aos Xenotrichulidae, se concentraram em descrições taxonômicas (e.g.Remane 1929, 1934, 1936, 1926, 1927; Roszczak 1939; Wilke 1954; D'Hondt 1966; Schrom 1966, 1972, Rao and Ganapati 1967, 1968; Ruppert 1979; Mock 1979; Kisielewski 1984, 1988; Rao 1991; Todaro 1994; Hummon and Todaro 2007) ou alguns trabalhos com foco no potencial críptico de uma de suas espécies e a estrutura muscular dos gêneros (e.g. Todaro et al. 1996; Hochberg and Litvaitis 2001; Leasi and Todaro 2009). O principal estudo sobre a família foi desenvolvido por Ruppert (1979). Neste estudo ele sumarizou todo o conhecimento produzido até aquela data sobre xenotrichulídeos e reorganizou todo o táxon. A partir desse trabalho, o gênero *Xenotrichula* é caracterizado, principalmente, como espécies portadoras de cílios locomotores de tamanhos iguais, região cefálica composta por dois ou três pares de cerdas sensoriais, um par de longos tentáculos laterais e presença de escamas pedunculadas do tipo “intermedia”. o gênero *Heteroxenotrichula* é caraterizado principalmente pela presença de cílios locomotores de tamanhos diferentes, região cefálica com dois pares de cerdas sensoriais, um pequeno tentáculo lateral e presença de escamas simples ou pedunculadas do tipo “subterranea” e furca pedunculada. *Draculiciteria* é reconhecido como espécimes que possuem uma pleura expandida, um cefálion bem desenvolvido, por um conjunto escamas simples dorso e ventralmente e escamas hidrofólicas lateralmente, por sua ciliação locomotora restrita a região da faringe e por ter 5/6 de sua furca coberta por escamas. Entretanto, até hoje, nenhum estudo filogenético foi realizado para demonstrar as relações entre os seus representantes e confirmar a monofilia dos gêneros e subgêneros da família Xenotrichulidae.

Objetivos

- Investigar as relações internas da Família Xenotrichulidae;
- Propor uma nova classificação dos gêneros a partir da análise filogenética;
- Investigar o potencial críptico das espécies da Família Xenotrichulidae;
- Descrever uma nova espécie de Xenotrichula encontrado no Brasil.

Materiais e Métodos

Pontos de coleta

Ao longo desse estudo, 30 diferentes praias foram visitadas e amostradas, 16 ao longo da costa brasileira (Fig 2A) e 16 ao longo da costa estadunidenses (Figs. 2B-C) (Tabela1). As praias brasileiras amostradas são caracterizadas como praias de média-alta energia e as estadunidenses como de baixa energia. Apenas uma amostra é oriunda de profundidade acima de 3 metros, Fort Pierce – Florida.

Análise Morfológica

Os gastrótricos foram extraídos do sedimento com uma solução isotônica de cloreto de magnésio 7% usando a técnica de anestesia-decantação (Pfannkuche & Thiel 1988) em frascos plásticos de Erlenmeyer de 1L. Os animais foram decantados em redes de 35-43 µm e lavados com água salina (coletada do ponto de coleta e filtrada em filtros de papel) em placas de Petri. Os epécimes foram observados sob o microscópio estereoscópico Leica EZ4 (EUA) e Zeiss Stemi 2000 (Brasil), separados e montados em lâminas para observação em vivo pelo microscópio composto Zeiss A1 DIC equipado com a câmera digital Sony Handycam (EUA), e Zeiss Axio Imager M2 equipado com DIC e câmera digital AxioCam MRC5. As posições anatômicas dos caracteres é fornecida através de unidades percentuais (U) do comprimento total do corpo (Hummon et al. 1992). Os registros digitais da nova espécie serão depositados no Museu de Zoologia da Universidade Estadual de Campinas.

Microscopia Confocal

Microscopia confocal foi utilizada para analisar a musculatura dos indivíduos de diferentes populações de *Xenotrichula intermedia*, coletados no Estado de New Hamphire, nos Estados Unidos.

Os espécimes foram fixados em paraformaldeído 4% em 0.1M de tampão salino fosfatado (pH 7.2) por pelo menos uma semana. Os indivíduos foram lavados em PBS e corados em Alexa Fluor 488 phalloidin (Life Technologies). Os espécimes corados foram lavados brevemente em PBS e montados em Fluoromount G (Southern Biotechnology Associates, Birmingham, AL) sobre lâminas vidro. O microscópio confocal Olympus FV 300 foi utilizado para observar os espécimes. Ar argon laser (488 nm) foi utilizado para reagir com as amostras e o software fornecido pelo fabricante Olympus foi usado para capturar as imagens. Os arquivos obtidos foram tratados e processados usando o software Volocity (Perkin Elmer) para gerar projeções-z.

Microscopia Eletrônica de Varredura (MEV)

Para obtenção de caracteres morfológicos foi utilizado o MEV. Para isso, foram utilizados duas técnicas de preparação:

Ponto crítico – os espécimes foram fixados em glutaraladeido 2.5% em 0.1 M de tampão de cacodilato sódico (pH 7.4) por diversos dias antes do processamento. Após a fixação os espécimes foram lavados em 0.1 M de PBS por 1 hr, pós-fixado em 1% OsO_4 em tampão 0.1 M por 1 hr, e então lavados novamente em tampão para serem submetidos à série de desidratação alcoólica. Em seguida os indivíduos foram subtmetidos ao ponto crítico de desidratação(CPD) usando equipamento de CPD, Tousimis Samdri-795 e coberto com ouro usando o *sputter coater* Denton Vacuum Desk IV e examinado sob o microscópio Amray 1400 SEM na Universdade de Massachusetts Lowell.

HMDS (hexamethyldisilazane) - os espécimes foram fixados em 2.5% glutaraldeído em 0.1M tampão de cacodilato sódico. Após a fixação os espécimes foram lavados em 0.1 M PBS por 1 hr, submetidos a série de desidratação alcoólica e tratados com HMDS (Hochberg & Litvaitis 2000) e então cobertos com uma liga de ouro-paládio. Os indivíduos foram examinados sob JSM 5800LV SEM na Universidade de Campinas (UNICAMP).

Tabela 1 – Coordenadas dos pontos de coleta visitados ao longo do presente trabalho.

Latitude	Longitude	Cidade	Estado	País
42°52'39.88"N	70°48'56.21"W	SeaBrook	New Hampshire	EUA
42°53'59.13"N	70°48'38.12"W	Hampton	New Hampshire	EUA
42°54'26.14"N	70°48'34.74"W	Hampton	New Hampshire	EUA
42°55'58.84"N	70°47'48.65"W	Hampton	New Hampshire	EUA
42°57'0.35"N	70°47'8.79"W	Hampton	New Hampshire	EUA
43°10'30.90"N	70°36'30.90"W	York	Maine	EUA
43°14'13.58"N	70°35'22.92"W	Ogunquiit	Maine	EUA
42°47'16.15"N	70°48'21.74"W	Newbury	Massachussetts	EUA
27°27'40.39"N	80°18'46.13"W	Fort Pierce	Florida	EUA
27°28'47.40"N	80°13'41.40"W	Fort Pierce - Off Shore	Florida	EUA
27°21'58.28"N	80°14'47.86"W	Green Turtle Beach	Florida	EUA
27°35'11.29"N	80°19'42.55"W	Vero Beach	Florida	EUA
27°35'26.32"N	80°19'45.89"W	Vero Beach	Florida	EUA
27°39'13.16"N	80°21'17.83"W	Vero Beach	Florida	EUA
23°48'17.08"S	45°23'48.90"W	São Sebastião	São Paulo	Brasil
23°38'22.3"S	45°25'16.1"W	Caraguatatuba	São Paulo	Brasil
22°31'57.15"S	41°57'23.20"W	Rio das Ostras	Rio de Janeiro	Brasil
22°31'41.50"S	41°56'44.96"W	Rio das Ostras	Rio de Janeiro	Brasil
22°23'50.83"S	41°47'9.46"W	Macaé	Rio de Janeiro	Brasil
22°23'25.47"S	41°46'21.15"W	Macaé	Rio de Janeiro	Brasil
22°20'2.75"S	41°44'0.15"W	Macaé	Rio de Janeiro	Brasil
17°54'24.58"S	39°22'3.51"W	Nova Viçosa	Bahia	Brasil
17°54'4.60"S	39°21'35.09"W	Nova Viçosa	Bahia	Brasil
17°53'48.18"S	39°21'47.54"W	Nova Viçosa	Bahia	Brasil
17°53'43.79"S	39°21'49.08"W	Nova Viçosa	Bahia	Brasil
17°55'10.13"S	39°24'9.66"W	Nova Viçosa	Bahia	Brasil
17°59'37.84"S	39°29'18.90"W	Nova Viçosa	Bahia	Brasil
20° 1'56.51"S	40° 9'33.69"W	Nova Almeida	Espírito Santo	Brasil
20° 1'27.87"S	40° 9'30.91"W	Nova Almeida	Espírito Santo	Brasil
19°54'43.22"S	40° 5'51.19"W	Nova Almeida	Espírito Santo	Brasil

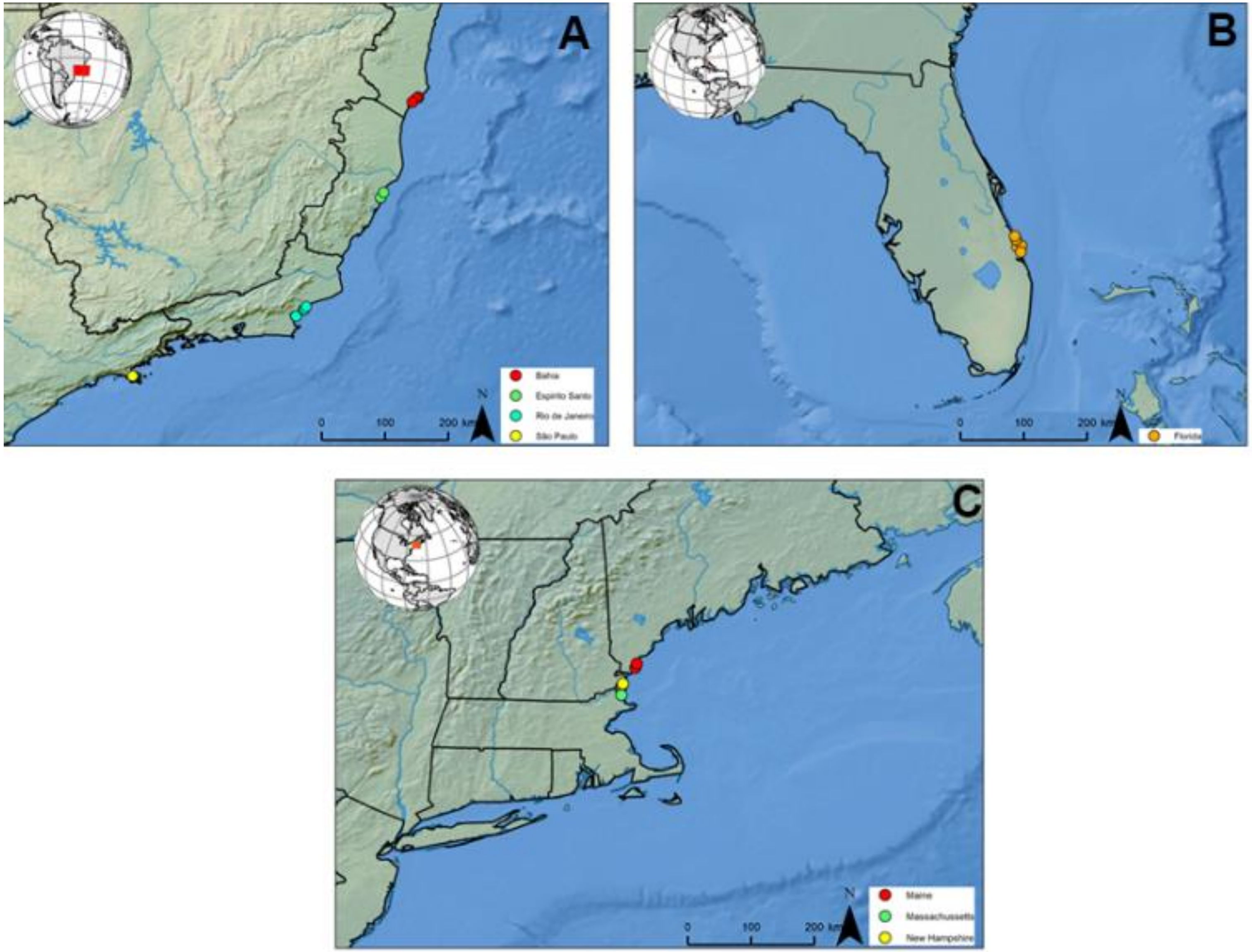


Figura 2 – Mapa esquemático dos pontos de coleta do presente estudo. A – Pontos da costa brasileira. B-C, pontos da costa estadunidense. B- Florida. C – Estados do norte da costa leste.

Resultados

Essa tese está organizada em três capítulos, que serão base para três artigos científicos que serão submetidos a revistas especializadas .

I – Xenotrichulidae (Gastrotricha: Chaetonotida): a morphological, phylogenetic, and systematic revision with a reclassification of genus-group based on tree thinking

Nesse capítulo será proposta uma nova classificação de toda a família Xenotrichulidae baseada em uma análise filogenética morfológica e uma padronização dos termos morfológicos descritos. Pela primeira vez, em um grupo de gastrótricos, será proposta uma classificação baseada em uma hipótese filogenética.

II - Chapter II – Evolution of the muscular system in family Xenotrichulidae (Gastrotricha: Chaetonotida) reveals 2 candidate species of *Rotunduxenotrichula intermedia*

No presente estudo, investigamos a organização do sistema muscular em *Rotunduxenotrichula intermedia* dos EUA e comparamos esses resultados com espécimes do Kuwait e da Itália para determinar se a organização muscular mostra evidências de diferenças entre espécies potencialmente crípticas. A maioria das diferenças entre as musculaturas dos espécimes analisados estava presente no número e na disposição dos músculos dorsoventrais e na presença de grande número de músculos circulares somáticos. Nossos resultados reforçam a hipótese de que *R. intermedia* representa um grupo de espécies crípticas formado por pelo menos duas espécies candidatas.

III –Descrption of *Xenotrichula* sp. nov. (Gastrotricha: Xenotrichulidae) from Brazilian coast

O capítulo trará a descrição de uma nova espécie do gênero *Xenotrichula* encontrada nos Estados de São Paulo e Bahia

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Referências

Araújo T.Q., Atherton S., Hochberg R. 2015. First record and a description of a new species of *Oregodasys* (Gastrotricha: Macrodasyida: Thaumastodermatidae) from Tobago. Proc. Biol. Soc. Washingt. 128:190–199.

Balsamo M., D`Hondt J.-L., Kisielewski J., Todaro M., Tongiorgi P., Guidi L., Grilli P., de Jong Y. 2015. Fauna Europaea: Gastrotricha. Biodivers. Data J. 3:e5800.

Balsamo M., Guidi L., D'Hondt J.L. 2013. Phylum Gastrotricha. Zootaxa. 3703:79–82.

Balsamo M., Todaro M.A. 2002. Gastrotricha. In: Rundle S.D., Robertson A.L., Schmid-Araya J.M., editors. Freshwater Meiofauna: Biology and Ecology. Leiden: Backhuys Publishers. p. 45–61.

D'Hondt J.L. 1966. Chaetonotoides marins et d'eau douce de la région d'Arcachon. Actes la Société Linnéenne Bordeaux. 103A:1–22.

Ehrenberg C.G. 1830. Organisation, systematik und geographisches verhältniss der infusionsthierchen. Zwei vorträge, in der Akademie der wissenschaften zu Berlin gehalten in den jahren 1828 und 1830, von C.G. Ehrenberg. Mit 8 kupfertafeln in folio ... Berlin,: Druckerei der Königlichen akademie der wissenschaften,.

Garraffoni A.R.S., Araújo T.Q., Lourenço A.P., Guidi L., Balsamo M. 2016a. A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. Syst. Biodivers.

Garraffoni A.R.S., Di Domenico M., Hochberg R. 2016b. New records of marine Gastrotricha from São Sebastião Island (Brazil) and the description of a new species. Mar. Biodivers.:1–9.

Hochberg R., Litvaitis M.K. 2001. The musculature of *Draculiciteria tessalata* (Chaetonotida, Paucitubulatina): implications for the evolution of dorsoventralmuscles in Gastrotricha. Hydrobiologia. 452:155–161.

Hummon W.D., Todaro M.A. 2007. A new species of Xenotrichulidae (Gastrotricha) from southern and southeastern USA. Cah. Biol. Mar. 48:297–302.

Hummon WD, Balsamo M, Todaro MA (1992) Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. Bolletino di Zool 59:499–516. doi: 10.1080/11250009209386711

Kånneby T. 2016. Phylum Gastrotricha. Thorp and Covich's Freshwater Invertebrates. Elsevier. p. 115–130.

Kieneke A., Riemann O., Ahlrichs W.H. 2008. Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. Zool. Scr. 37:429–460.

Kieneke A., Schmidt-Rhaesa A., Hochberg R. 2015. A new species of *Cephalodasys* (Gastrotricha, Macrodasyida) from the Caribbean Sea with a determination key to species of the genus. Zootaxa. 3947:367.

Kisielewski J. 1984. *Xenotrichula guadelupense* n.sp., a new marine gastrotrich from Guadeloupe. Bull. du Muséum Natl. d'Histoire Nat. Paris. 6A:37–40.

Kisielewski J. 1988. New records of marine gastrotricha from the French coasts of Manche and Atlantic .2. Chaetonotida, with description of 4 new species. Cah. Biol. Mar. 29:187–214.

Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. Invertebr. Syst. 30:398.

Kolicka M., Janikowska E., Kotwicki L. 2015. Baltic Sea Gastrotricha—one new species and one new record of Chaetonotida from Poland. Zootaxa. 4027:487.

Leasi F., Todaro M.A. 2009. Meiofaunal cryptic species revealed by confocal microscopy: the case of *Xenotrichula intermedia* (Gastrotricha). Mar. Biol. 156:1335–1346.

Mecnikow E. 1865. Ueber einige wenig bekannte niedere Thierformen. Zeitschrift für wissenschaftliche Zool. 15:450–463.

Mock H. 1979. Chaetonotoidea (Gastrotricha) der Nordseeinsel Sylt. Mikrofauna des Meeresbodens. 78:403–507.

Müller O.F. 1773. Vermium terrestrium et fluviatilium, seu animalium infusoriorum, helminthicorum et testaceorum, non marinorum, succincta historia.

Pfannkuche O, Thiel H. 1988. Sampling processing. *Introduction to the Study Meiofauna*.134–145.

Rao G.C. 1991. Meiofauna. Calcutta: Zoological Survey of India.

Rao G.C., Ganapati P.N. 1967. On some interstitial polychaetes from the beach sands of Waltair Coast. Proc. Indian Acad. Sci. - Sect. B. 66:10–15.

Rao G.C., Ganapati P.N. 1968. Some new interstitial gastrotrichs from the beach sands of Waltair coast. Proc. Indian Acad. Sci. - Sect. B. 67:35–53.

Remane A. 1926. Marine Gastrotrichen aus der Ordung der Chaetonotoidea. Zool. Anz. 66:243–252.

Remane A. 1927. *Xenotrichula velox* nov. gen. nov. spec., ein chaetonotoides Gastrotrich mit mäennlichen Geschlectsorganen. Zool. Anz. 71:289–294.

Remane A. 1929. Gastrotricha. In: Kükenthal W., Krumbach T., editors. Handbuch der Zoologie. Berlin: Walter de Gruyter. p. 121–186.

Remane A. 1934. Die Gastrotrichen des Küestengrundwassers von Schilksee. Schr. Naturwiss. Ver. Schleswig-Holstein. 20:473–478.

Remane A. 1936. Gastrotricha. In: Bronn H.G., editor. Klassen und Ordnungen des Tierreichs. Leipzig: Akademische Verlagsgesellschaft. p. 1–242.

Roszczak R. 1939. Die Psammitgastrotricha des polnischen Ostseestrandes. Zool. Pol. 4:1–24.

Ruppert E.E. 1979. Morphology and systematics of the Xenotrichulidae (Gastrotricha, Chaetonotida). Mikrofauna des Meeresbodens. 76:239–292.

Schrom H. 1966. Gastrotrichen aus Feinsanden der Umgebung von Venedig. Boll. del Mus. Civ. di Stor. Nat. di Venezia. 17:31–45.

Schrom H. 1972. Nordadriatische Gastrotrichen. Helgoländer Wissenschaftliche Meeresuntersuchungen. 23:286–351.

Todaro M.A. 1994. *Chaetonotus triacanthus* and *Heteroxenotrichula texana*, Two New Chaetonotid Gastrotrichs from the Gulf of Mexico. Trans. Am. Microsc. Soc. 113(1):15–21.

Todaro M.A., Fleeger J.W., Hu Y.P., Hrincevich A.W., Foltz D.W. 1996. Are meiofaunal species cosmopolitan? Morphological and molecular analysis of *Xenotrichula intermedia* (Gastrotricha: Chaetonotida). Mar. Biol. 125:735–742.

Wilke U. 1954. Mediterrane Gastrotrichen. Zool. Jahrbücher Abteilung für Syst. Ökologie und Geogr. der Tiere. 82:497–550.

Zelinka C. 1889. Die Gastrotrichen. Eine Monographische Darstellung ihrer Anatomie, Biologie und Systematik. Zeitschrift für wissenschaftliche Zool. 49:209–384.

Chapter I - Xenotrichulidae (Gastrotricha: Chaetonotida): a morphological, phylogenetic, and systematic revision, with a genus-group reclassification based on tree thinking[1]

Abstract.

The marine Xenotrichulidae is composed of species characterized by tenpin-shaped bodies with ventral locomotory cirri restricted on the pharynx region and a pair of tufts on the ventral middle body. The last revision of the family was performed more than 35 years ago and, since then, 13 new species were described. The present study provides a phylogenetic reconstruction of the Xenotrichulidae including a deep analysis that sought a new standardization of some morphological terms and a new interpretation of character homologies using modern morphological techniques. The phylogenetic analysis of xenotrichulids obtained by parsimony analysis was used as backbone to propose a new classification that reflects these novel relationships. This research is therefore the first attempt to link traditional Linnean ranks (family and genus-groups) with phylogenetic relationships based on the possession of shared characters (apomorphies) to determine monophyly at the lowest taxonomic levels. Within the family Xenotrichulidae, three subfamilies are recognized: Draculiciterinae Ruppert, 1979 is the sister-group of Xenotrichulinae and *Rotunduxenotrichulinae* **subfam. nov.** Within Draculiciterinae, the monotypic genus *Draculiciteria* is reconignized, which is composed solely o*f D. tesselata*. Within, Xenotrichulinae, two genera are recognized: *Minutuxenotrichula* **gen. nov.,** which includes *M. tentaculata* **nov. comb.** and *M. laccadivensis* **nov. comb.,** and *Xenotrichula* **nov. comb**., which contains *Xenotrichula velox*, *X. guadelupensis*, *X. cornuta*, *X.* sp. nov., *X. texana* **nov. comb.,** *X. simplex* **stat. rev.,** *X. transatlantica* **nov. comb.**, *X. wilkeae* **nov. comb.**, *X. affinis* **stat. rev.**, *X. arcassonensis* **nov. comb.,** *X. subterranea* **stat. rev.**, *X. squamosa* **nov. comb.** and *X. pygmaea* **stat. rev.** Within Rotunduxenotrichulinae **subfam. nov.,** the genus *Rotunduxenotrichula* **gen. nov.** is recognized and includes *R. intermedia* **nov. comb.,** *R. quadritubulata* **nov. comb.,** *R. paralineata* **nov. comb.,** *R. lineata* **nov. comb.,** *R. floridana* **nov. comb.,** *R. soikai* **nov. comb.,** *R. micracantha* **nov. comb.,** *R. carolinensis* **nov. comb.,** *R. punctata* **nov. comb.** and *R. bispina* **nov. comb.** Complete taxonomic remarks are made for each species of Xenotrichulidae including notes on their global distribution patterns and identification keys for subfamilies, genera and species.

Keywords: Phylogeny; Gastrotricha; tree-thinking; reclassification; geographic distribution.

[1] Este capítulo está formatado de acordo com as normas fornecidas pela Revista PeerJ.

Introduction

Gastrotricha is a group of free-living microinvertebrates present in freshwater, estuarine and marine environments (Balsamo & Todaro 2002). The phylum includes about 820 species (Araújo, Atherton & Hochberg, 2015; Kieneke, Schmidt-Rhaesa & Hochberg, 2015; Balsamo et al., 2015; Kolicka, Janikowska & Kotwicki, 2015; Garraffoni, Di Domenico & Hochberg, 2016; Kånneby, 2016; Kolicka et al., 2016; Kolicka, 2017) grouped into two Linnean orders, Macrodasyida and Chaetonotida (but see Kieneke et al. 2008). The order Chaetonotida comprises about 450 species with a tenpin-shaped body, “Y”-shaped pharyngeal lumen, hermaphroditic reproductive system, and in many species, parthenogenesys. Two-thirds of the species of this group lives in freshwaters habitats (Balsamo & Todaro, 2002; Balsamo, Guidi & D’Hondt, 2013; Balsamo et al., 2015; Garraffoni et al., 2016).

Belonging to the order Chaetonotida Gosse, 1864, the marine family Xenotrichulidae was originally established by Remane (1927), in a brief description, when he described the new genus and new species *Xenotrichula velox* to accommodate specimens that present a pattern of restricted ventral ciliation, pedunculate scales on the dorsal cuticle, and paired testes. After his initial study, Remane (1934) described another four new species within *Xenotrichula*: *X. subterranea*, *X. affinis*, *X. pygmea* and *X. intermedia*. Also described the reproductive structures (e.g. eggs, ovaries, testes) and reported a new scales type, called hydrofoil scales, possibly related to their hydrodynamic potential during locomotion. However, only almost ten years after the original description of the family, Remane (1936) established a detailed family diagnosis.

In 1954, Wilke reexamined the genus *Xenotrichula* and established the new genus *Hetroxenotrichula* to accomodate a subset of the previsioly described species. This new genus included specimens with non-pedunculate scales and ventral locomotory cirri of different sizes. In the same study, Wilke (1954) also described the sperm morphology of four species and reported for the first time the presence of biflagellate spermatozoa in gastrotrichs.

It was not until the 1960´s and 1970´s that additional species of Xenotrichulidae were described, thereby increasing knowledge of the diversity within the family (e.g. D’Hondt 1966, 1968, Renaud-Mornant, 1968, Schrom 1966, 1972; Luporini et al. 1971). One of these studies was performed by Renaud-Mornant (1968), who discovered an unusual xenotriculid from Naples Bay (Italy). This new species, originally named *Polymerurus tessalata*, possessed some unusual characters: a set of plates around a larger cephalion on the head, lateral scales with expansions, and an elongate caudal furca covered by small plates except for the posterior tip. However, Hummon (1974b) pointed out that the species described by Renaud-Mornant (1968) did not belong to the genus *Polymerurus*, and so he established a new genus (*Draculiciteria*) within the Xenotrichulidae.

Although these new studies increased the knowledge of the diversity and distribution of the xenotriculids across the globe, some problems regarding the lack of standard terminology to describe the morphological features also increased. Thus, Ruppert (1979) peformed the first revision of the family in order to: a) standardize the morphological nomenclature used in the description of xenotriculids, b) summarize taxonomical information of all known species, and c) emend each of the three xenotriculid genera. He included the following emendments: *Draculiciteria* contains species with expanded pleuria, well developed cephalion, a conspicuous set of dorsal and ventral body imbricating scales, and lateral hydrofoil scales; *Xenotrichula* is manly recognized by the presence of locomotory cilia of the same length, two or three pairs of sensorial cilia on the head, and the head can be rounded or possess long lateral tentacles; *Heteroxenotrichula* contains species with locomotory cirri of different lengths, two pairs of sensorial head cirri, a short lateral tentacle, and subterranea-type scales or simple scales covering the dorsal body. Furthermore, Ruppert (1979) subdivided the genus *Xenotrichula* in two groups: a) *intermedia,* characterized by a dorsal body covered with intermedia-type scales, three pairs of sensorial head cirri, and a simple furca in the end body; and b) *velox*, recognizable by the dorsal body covered by subterranea-type scales, two pairs of sensorial head cirri, a pair of long lateral tentacles, and a pedunculate furca on the end body. The author also divided the genus *Heteroxenotrichula* in two groups: a) *subterranea*, characterized by the presence of the dorsal and dorso-lateral body covered by subterranea-type scales and by the presence of an oval patch of transverse scales on the dorsal cephalic region; and b) *squamosa*, recognizable by the prcsence of simple scales covering the dorsal body.

Hummon and Todaro (2010) published an analytic review about the taxonomic status of the names of all known marine gastrotrichs, including 13 species of Xenotriculidae described after Ruppert´s revision. Within the genus *Xenotrichula*, these authors established two new subgenera, *Xenotrichula* and *Velox*, to accommodate, respectively, the so called *intermedia* and *velox* groups. However, Abukawa and Kajihara (2011) highlighted that the new classification proposed by Hummon and Todaro (2010) did not accurately follow article 44.1 of International

Code for Zoological Nomenclatures (ICZN, 1999), because the name-bearing type species of the genus, *Xenotrichula velox*, needs to be linked to the same subgeneric name of *Xenotrichula.*

Traditionally, the hierarchical systematization for most group of animals is based on external and internal morphology without any support from phylogenetic studies, which has led to the establishment of many non-monophyletic taxa. The phylogenetic analysis allows researchers to test the evolutionary relationships of similar taxa and derive a more natural classification (Vogt et al. 2010; Páll-Gergely 2017).

The present study attempts to assess the phylogenetic relationships within Xenotriculidae and provide a detailed discussion of characters based on morphological features. A morphological and distributional description of species of Xenotrichulidae is detailed here. Furthermore, an identification key will be provided by this survey.

Material and methods

Taxonomic list and geographic distribution

The review considers all Xenotrichulidae species based on published data and the data gathered in the present study. Some descriptions did not provide the geographic coordinates of the sampling site, but only the name of the city. In these cases, we used the geographic coordinates of the downtown beach using the Google Earth software. The species that were not formally described were not considered in the present work: *X.* sp. A, *X.* sp. B (Schmidt, 1974), *X*. sp. D (Hummon, 1974a), *H.* sp.1, *H.* sp.2 (Valboresi & Lupporini, 1987), *H.* sp.1, *X.* sp.1 (Todaro & Rocha, 2004) and *H.* sp.1 (Todaro & Rocha, 2005), *Xenotrichula* species France A, *Heteroxenotrichula* sp.Italy-A, *Heteroxenotrichula* sp.Egypt-A, *Xenotrichula* species Oregon 1, *Xenotrichula* species Oregon 2, *Heteroxenotrichula* Hawaii sp.A, *Xenotrichula* Hawaii sp.B, *Xenotrichula* Indonesia sp.A , *Xenotrichula* Oregon sp.A, *Xenotrichula* species England A (Hummon, 2010).

Spalding et al. (2007) defines three levels of marine biogeographical regions: Realms, Provinces and Ecorregions. The knowledge of boundaries of the marine environment is important to evaluate which environmental factors can influence the dispersion and colonization of the marine species. We will provide the species distributions of Xenotricullidae using the marine biogeographical realms (Fig. 1).

Ingroup taxa

Almost all formally described species within the family Xenotriculidae were used in the present analysis. Only *Xenotricula micracantha* was not used due to incomplete original description. We used 15 species of *Xenotricula* (one of these species is described in chapter 3 – *X.* sp. nov.), 9 species of *Heteroxenotricula* and 1 species of *Draculiciteria*.

Outgroup taxa

Some molecular analyses placed Xenotrichulidae as sister group of the family Chaetonotidae Gosse, 1864 (Paps & Riutort, 2012; Kånneby, Todaro & Jondelius, 2013), but other analyses (Bekkouche & Worsaae, 2016) placed Xenotrichulidae as sister group of Muselliferidae Leasi & Todaro, 2008. Thus, as outgroups we choose one species of Chaetonotidae (*Chaetonotus mariae* Todaro, 1992) and one species of Muselliferidae (*Diuronotus aspetos Todaro, Balsamo & Kristensen, 2005).*

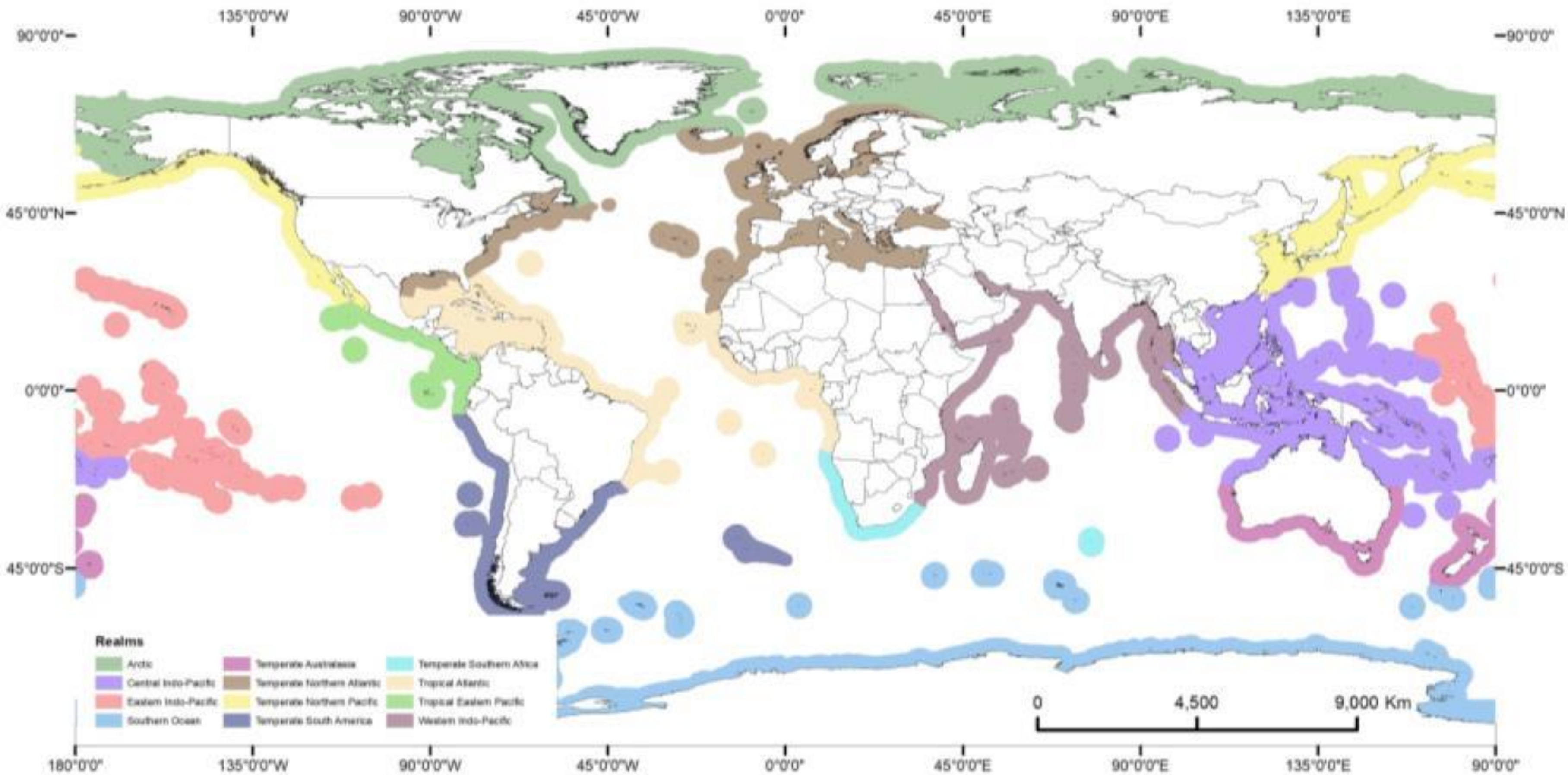

Figure 1 - Marine Realms proposed by Spalding et al. 2007

Scanning electron microscopy

For scanning electronic microscopy (SEM) analysis, we used two techniques:

Critical Point Drying: specimens were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for several days prior to processing. Specimens were then rinsed in 0.1 M buffer for 1 h, postfixed in 1% OsO_4 in 0.1 M buffer for 1 hr, and then rinsed again in buffer prior to a standard ethanol dehydration series. Specimens were next critical-point dried (Tousimis Samdri-795) and coated with gold using a sputter coater (Denton Vacuum Desk IV) prior to examination with an Amray 1400 SEM at the University of Massachusetts Lowell.

HMDS: specimens were fixed in 2% glutaraldehyde in cacodylate buffer or 2% paraformaldehyde in PBS, then rinsed in 0.1 M PBS, dehydrated through a graded ethanol series, treated with HMDS (hexamethyldysilazane) (Hochberg & Litvaitis 2000), mounted on aluminium stubs, and sputter coated with gold-palladium. Observations were carried out under a SEM JSM 5800LV, at the State University of Campinas.

Character coding and analysis

We coded 51 morphological characters. Most of them were coded as multistate or binary, but some were coded as contingent. Contingent coding (Forey & Kitching, 2000), also called C coding (Pleijel, 1995) or conditional coding (Hawkins, Hughes & Scotland, 1997) codes first a binary character, in one column of the matrix, and an additional code for presence and absence of the feature in the next column. Non-applicable states were coded as '–' and unknown states as '?'.

Parsimony analysis was carried out using the computer program TNT under equal and implied weights (Goloboff, Farris & Nixon, 2003, 2008) in order to perform a 'sensitivity analysis' of the data set under distinct situations. For equal weights we used exhaustive search. When weighting was used, the values of K were those that were assigned to a 'not perfectly hierarchical' character fit of 50%, 55.3%, 60.6%, 66%, 71.3%, 76.6%, 82%, 87.3%, 92.6% and 98% (script commands aaa 3 10 50 98 7) – all relative to the fit of a perfectly hierarchical one (regular intervals of 5.333%). Mirande (2009) proposed regular intervals fit/distortion values, which can be obtained under different K-values (appendix S3 of Mirande 2009 – file aaa.run). Branch support was evaluated using relative Bootstrap analysis (10000 replicates) and Bremer support. The software WINCLADA 1.00.08 (Nixon 2002) was used for character optimization and tree editing.

Morphological description of main characters

The complete character list can be found in table 1.

Cover of anteriormost dorsal region of head

Usually the term cephalion is used to name a specific plate on the dorsal region of the head. Some authors also described it as "undefined area" on the anteriormost head. We treat here the region of the anteriormost dorsal head as homologous among species of Xenotrichulidae. The anteriormost dorsal head can be covered by scales, plate(s) or naked (Figs. 3A-B).

Cover of central dorsal region of head

An oval patch composed by transversal plates extending from around U3 to U15, and can be present on the dorsal head. When this structure is absent, this area can be covered by the dorsal scales.

Cephalic sensorial tufts

Specimens of xenotrichulids present three arrangements of head sensorial tufts: two pairs of sensorial tufts, a) one rising at the top of the dorsal head and the second one at the lateral head, b) one rising at margin of dorsal head and the second one at the lateral head or c) three pairs of sensorial tufts (Fig. 3C).

Oral bristles

Present on each side of mouth, variable in number (Fig. 3C). The functions of these structures are unknown.

Tentacle

Some xenotrichulids present tentacles on the lateral region of head (Fig. 3A). The tentacles can be naked or covered by scales and/or cilia. Regarding the size, the tentacles can be treated as short or long. To define the size, we provide the following:

Long tentacles – at least 35% of the widest length of the head;

Short tentacles – maximum of 25% of the widest length of the head.

Cover of the dorsal lateral head

Different covering arrangements can be present on this region: Pleuria, *Draculiceteria*-type plates (Fig. 3D) and scales supported (or not) by a pedunculum. However, these plates are not considered homologous with the pleuria observed in Chaetonotidae specimens.

Cover of the ventral head

Just ventral to the mouth opening, a plate or a set of plates called the hypostomium (hypostomia) are present in *Draculiciteria tesselata* and some Chaetonotidae. In *Xenotrichula* and *Heteroxenotrichula*, this region can be covered by scales or be a naked area (Fig. 3D)

Hard projections on each side of mouth

The hypostomium is a plate or a set of plates on the ventral side of the head just posterior of the mouth (Fig. 3D). A pair of hard projections on each side of the mouth was also named as the hypostomium in xenotrichulids (Ruppert, 1979). These two kinds of structures are inserted on different body places. For a biological understand we define that the hypostomium and this projection are not the same structure.

Transversal row of cilia above mouth

A transversal row of cilia beginning on each side above the mouth extending to the tip of the tentacles at the ventral side (Fig. 3E). This character was here renamed in order to make a clear statement about its structure and its distribution and not just its shape ("Kammantiger Zacken" what means in a free translation from German language as “combed teeth”)

Dorsal scales

All xenotrichulids possess scales on the dorsal side of the body, but these scales have different shapes such as polygonal, oval, hexagonal, intermedia-type, or subterranean-type. These scales can be of identical size, increasing size along the body axis, or decreasing size at the end of the body. Some scales lay flat on the cuticular surface while other are pedunculated (stalked).

Dorsal sensorial bristles

Some specimens show sensorial bristles on the dorsal body wall. These bristles can be short or long and are distributed in different arrangements along the body. The furca can have sensorial bristles with different sizes. The following measures were used to define the transformation series of the character:

Short sensorial bristles - maximum 65% of the widest length of the trunk

Long sensorial bristles - at least 90% of the widest length of the trunk

Ventral scales

The ventral body can be naked or covered by scales. As on the dorsal side, ventral scales can be supported by a peduncle. The covering arrangement varies among xenotrichulids, e.g., restricted on the pharynx region; covering the entire ventral body; covering only the anterior part of the intestine until the locomotory tufts; restricted to the second half of the body.

Longitudinal plates on the ventral body

Two to four ventral longitudinal columns of plates on the central body from middle trunk to top of furca. These plates can be misinterpreted by a microscopic artifact. *Xenotricula.* sp. nov. (Chapter 3) presents four columns of plate under light microscopy but when observed on SEM only two rows are present (Fig. 3G).

Locomotory cirri

Most species of Chaetonotida possess ventral locomotory cilia composed of individual cilia that arise separately from the body and are each covered in their own cuticle. On the other hand, the xenotrichulids possess groups of cilia called cirri that are covered by a single continuous cuticle (Figs. 3D-E, G-H).

Locomotory cirri distribution on the pharynx region

Xenotrichulidae specimens present locomotory cirri in different arrangements on the ventral body: a) lateral parallel longitudinal columns extending along the pharynx length; b) anterior part of pharynx covered by cirri and dividing (U15-U18) into two lateral parallel longitudinal columns extending to the end of pharynx length, and c) entire pharynx region covered. Species with tentacles present the cirri in different sizes in this region.

Locomotory cirri arrangement on the intestine region

Xenotrichulids, except *Draculiciteria tesselata,* presents on the ventral middle trunk a pair of locomotory cirri tufts. A second pair of tufts can be present on the posterior ventral trunk (U79) of some species. This character is present only on species of Xenotrichulidae species.

Furca

The chaetonotid furca is usually composed by a short post-anal base and paired adhesive tubes. Xenotrichulidae species presents a furca composed by a long post-anal base and short adhesive tubes. The base is covered by flat scales and length of the base can throughout the family: 1/3, 2/3 or ½ of total length of furca.

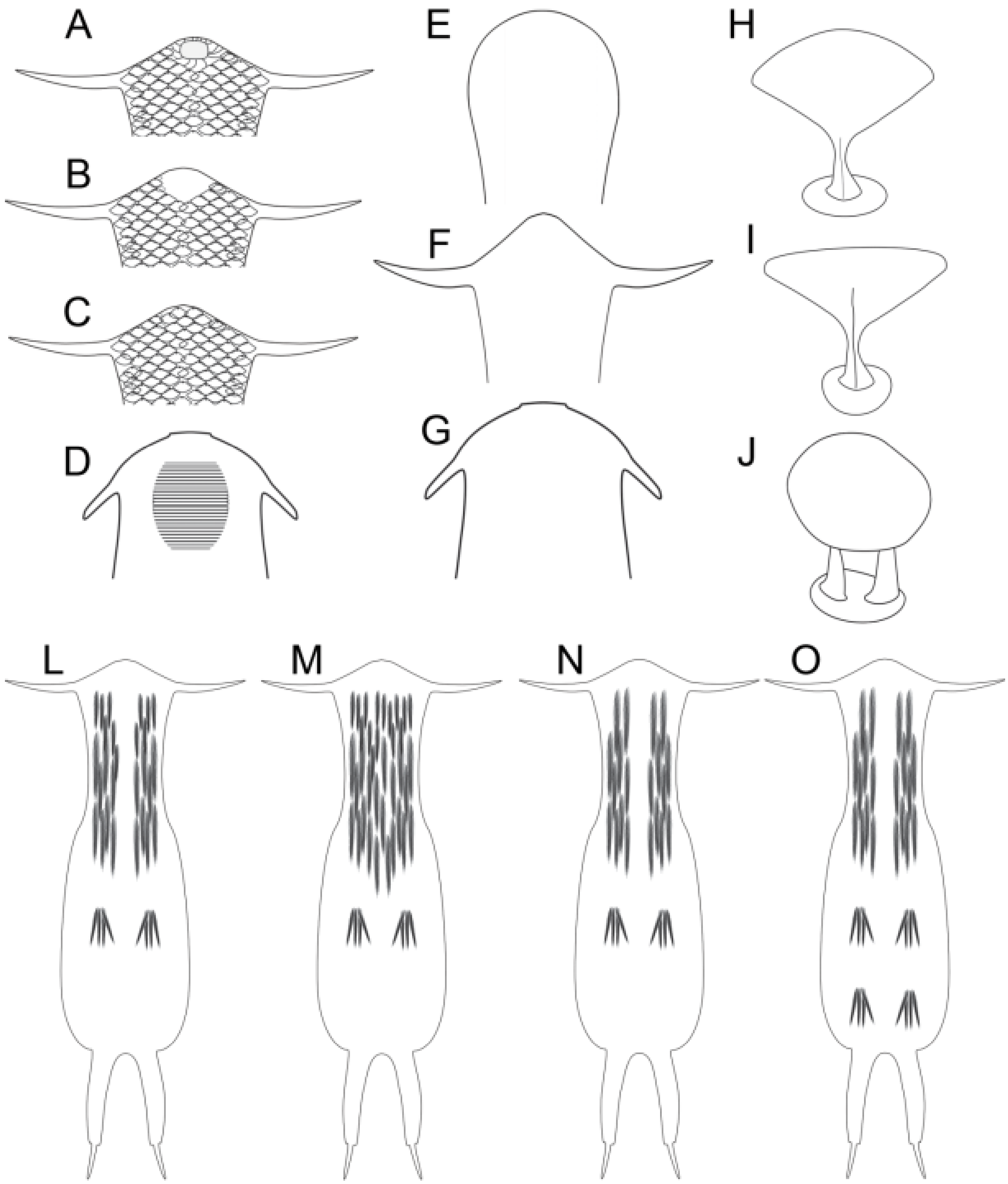

Figure 2 – Schematic draw of morphological features. A – C, different arrangements of cover of cephalion region: A - Plate; B – Naked area; C – scales. D – Oval patch on the central head. E – G, head shapes: E – Rounded; F; Long lateral tentacles; G, Short lateral tentacles. H – J, different scales supported by pedunculum, H, subterranea-type; I, Intermedia-type; J, Hexagonal supported by two pedunculua. L – O, different arrangement and size of ventral locomotority cirri: L, different sizes arranged in two parallel rows on the pharynx length and two locomotry cirri tufts on middle trunk; M, different sizes covering all pharynx regions and two locomotry cirri tufts on middle trunk; N, different sizes arranged in two parallel rows on the pharynx length and two locomotry cirri tufts on middle trunk; different sizes arranged in two parallel rows on the pharynx length, two locomotry cirri tufts on middle trunk and two pairs on posterior trunk. Light grey refers to the small locomotory cirri; Dark grey refers to the large locomotory cirri

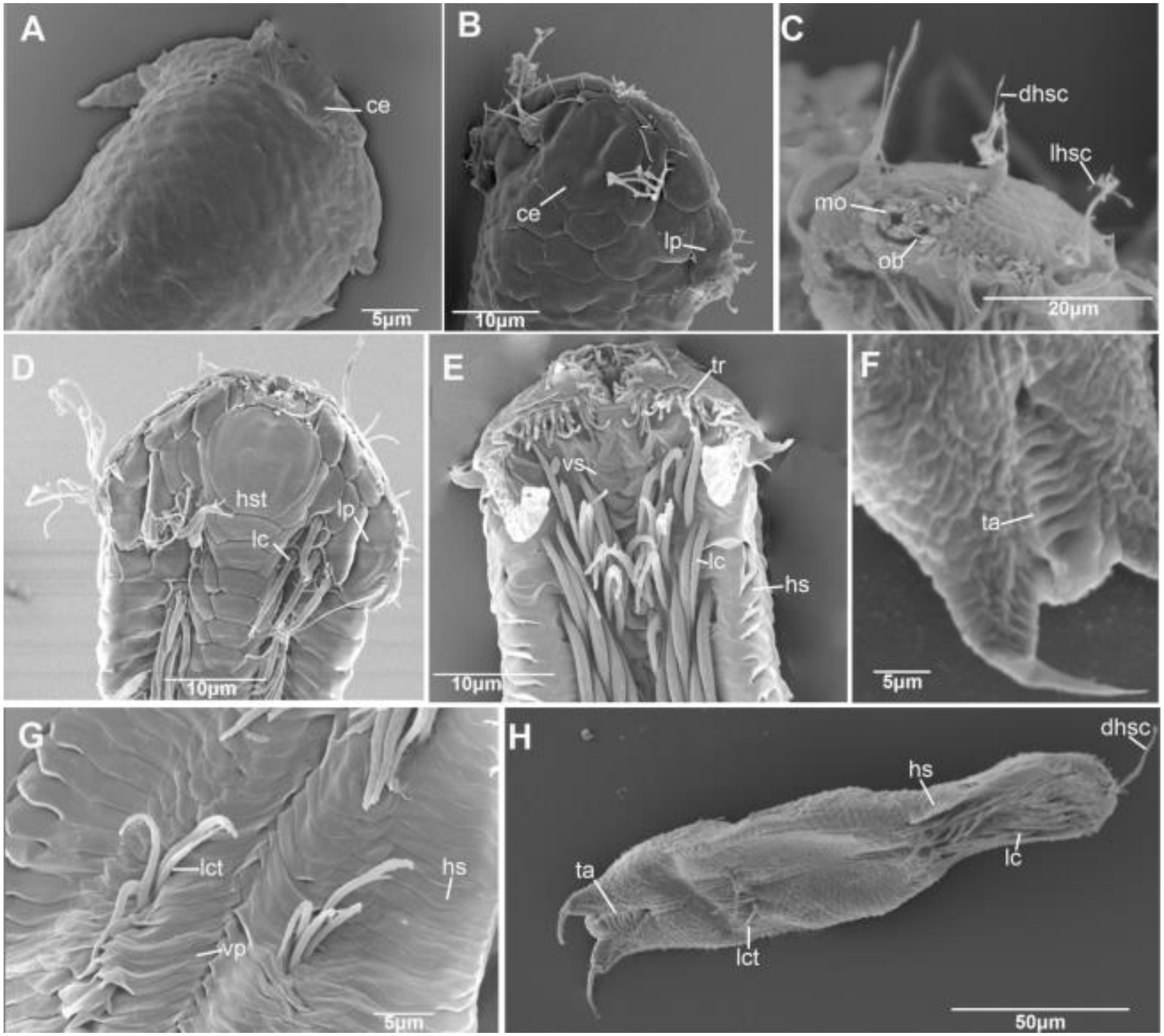

Figure 3 - SEM photographies. A, Dorsal head of *X. tropicallis*; B, dorsal head of *D. tesselata*; C, dorso-ventral view of head of *X. intermedia*; D, ventral view of *D. tesselata;* E, ventral view of *X. tropicallis*; F, ventral view of end body of *X. intermedia*; G, ventral view of middle trunk of *X. tropicallis*; H, ventral view of entire body of *X. intermedia.* Abbreviations: ce, cephalion; dhsc, dorsal head sensorial cirri; hs, hydrofoil scales; hst, hypostomium; lc, locomotory cirri; lct, tufts of locomotory cirri on the middle ventral trunk; lhsc, lateral head sensorial cirri; lp, lateral head plates; mo, mouth; ob, oral bristles; ta, triangular area of flat plates; tr, transversal row of cilia; vp, ventral plates; vs, ventral scales

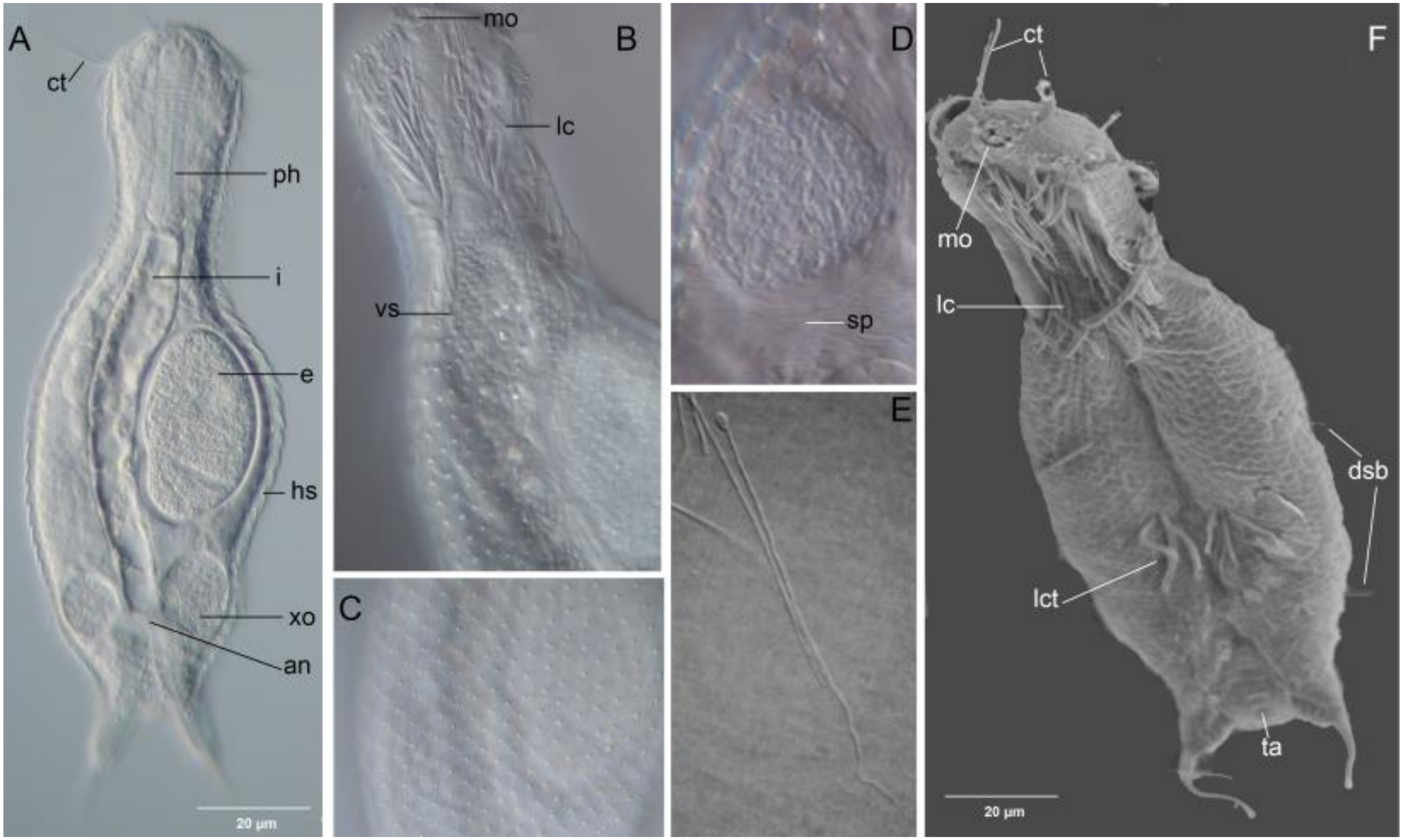

Figure 4 - A-G, *Xenotrichula intermedia*. A. Internal body; B. Ventral first half body. C. Close-up to intermedia-type scales on dorsal trunk. D. Close-up on X-organ content. E. Single sperm. F. SEM photography of ventral body. Abbreviations: an, anus; ct, cephalilic sensorial tufts; dsb, dorsal sensorial bristles; e, egg; hs, sydrofoil scales; I, intestine; lc, locomotory cirri; lct, locomotory cirri tufts; mo, mouth; xo, x-organ.

Table 1 - Characters coded in the present analysis

0. Cephalion
   - 0- Absent
   - 1- Present
1. Cover of anteriormost dorsal head
   - 0 - small plate
   - 1 - flat scales
   - 2 - scales with pedunculum
   - 3 - naked
   - 4 - keeled scales
2. Cephalion plate surrounded by a set of plates
   - 0 - Absent
   - 1 - Present
3. Hypostomium plate surrounded by a set of plates
   - 0 - Absent
   - 1 - Present
4. Cover of central dorsal head area (U3-U15)
   - 0 - scales
   - 1 - scales with pedunculum
   - 2 - transversal plates
   - 3 - transversal plates with three edges
   - 4 - keeled scales
5. Insertion of cephalic sensorial tufts.
   - 0 - Lateral
   - 1 - Dorsal + lateral
   - 2 - Dorsal
6. Number of cephalic sensorial tufts
   - 0 - 1 pair
   - 1 - 2 pairs
   - 2 - 3 pairs
7. Oral bristles
   - 0 - Absent
   - 1 - Present
8. Tentacle
   - 0 - Absent
   - 1 - Present
9. Cover of tentacle
   - 0 - Naked
   - 1 - Scales along entire length and short bristles on the tips
   - 2 - Scales and bristles along entire length
   - 3 - Scales along entire length
10. Tentacle size
    - 0 - Short
    - 1 - Long
11. Cover of the dorsal lateral head
    - 0 - Pleura
    - 1 - Draculiceteria-type plates
    - 2 - scales with pedunculum
    - 3 - scales without pedunculum
12. Cover of the ventral head
    - 0 - Naked

1 - Hipostomium
2 - scales without pedunculum
3 - scales with pedunculum

13. A pair of hard projections on each side of mouth
    0 - Absent
    1 - Present
14. Transversal row of cilia above mouth (old "Kammantiger Zacken")
    0 - Absent
    1 - Present
15. Dorsal scales shape
    0 - rounded
    1 - intermedia-type
    2 - subterranea-type
    3 - hexagonal
    4 - elliptical
    5 - oval
    6 - polygonal
16. Pedunculum supporting scales
    0 - Absent
    1 - Present
17. Additional pedunculum supporting scales
    0 - Absent
    1 - Present
18. Dorsal scale size along the body
    0 - Same size
    1 - Slightly increasing along the body
    2 - Abruptly increasing along the body
    3 - Same size until the end body, where are
19. Spines on dorsal scales
    0 - Absent
    1 - Present
20. Sensorial bristles on the dorsal body
    0 - Absent
    1 - Present
21. Size of sensorial bristles on the dorsal body
    0 - Short
    1 - Long
22. Pair of sensorial bristles on the dorsal head
    0 - Absent
    1 - Present
23. Additional pair sensorial bristles on the dorsal head
    0 - Absent
    1 - Present
24. Pair of sensorial bristles on the dorsal neck
    0 - Absent
    1 - Present
25. Additional pair sensorial bristles on the dorsal head
    0 - Absent
    1 - Present
26. Pair sensorial bristles on the anterior dorsal trunk
    0 - Absent

1 - Present

27. Additional pair sensorial bristles on the anterior dorsal trunk
    0 - Absent
    1 - Present
28. Pair sensorial bristles on the middle dorsal trunk
    0 - Absent
    1 - Present
29. Additional pair sensorial bristles on the middle dorsal trunk
    0 - Absent
    1 - Present
30. Pair sensorial bristles on the posterior dorsal trunk
    0 - Absent
    1 - Present
31. Additional pair sensorial bristles on the posterior dorsal trunk
    0 - Absent
    1 - Present
32. Pair sensorial bristles on the furca
    0 - Absent
    1 - Present
33. Additional pair sensorial bristles on the furca
    0 - Absent
    1 - Present
34. Modified spines
    0 - Absent
    1 – Rearmost lateral body
    2 – Intrafurcal region
35. Ventral scales
    0 - Absent
    1 - Present
36. Ventral scales distribution
    0 - Restrict to pharynx length
    1 - Restrict to intestine region, entire covered to the locomotry cirri tufts and dividing in two lateral portion to the top of furca
    2 - Restrict to intestine region, entire covered by four rows and dividing in two lateral portion to the top of furca
    3 - Entire ventral body
    4 - Entire second half body
37. Cover of ventral field
    0 - absent
    1 - scales without pedunculum
    2 - scales with pedunculum
38. Cover of lateral portion of ventral body (lateral of ventral field)
    0 - absent
    1 - scales without pedunculum
    2 - scales with pedunculum
39. Ventrolateral scales
    0 - scales with pedunculum
    1 - scales without pedunculum
    2 – Hydrofoil scales
40. Longitudinal plates on the ventral body
    0 - Absent

1 - Present

41. Triangular area of flat scales on the ventral end body
    0 - Absent
    1 - Present

42. Triangular area of spines on the ventral end body
    0 - Absent
    1 - Present

43. Locomotory cirri (arrangement)
    0 - Individual cilia rising from the body
    1 - a group of cirri rising together from the body

44. Locomotory cirri distribution on the pharynx region
    0 - lateral parallel longitudinal bands
    1 - anterior part of pharynx covered by cirri and dividing (U15-U18) in two lateral parallel longitudinal bands extending to the end of pharynx
    2 - Entire pharynx region covered

45. Lateral parallel longitudinal bands of locomotry cirri on the intestine region
    0 - Absent
    1 - Present

46. Pair of locomotory cirri tufts on the middle trunk
    0 - Absent
    1 - Present

47. Pair of locomotory cirri tufts on the posterior trunk (U79)
    0 - Absent
    1 - Present

48. Size of locomotory
    0 - Equal size
    1 - Different size on the pharynx cirri
    2 - Different size between pharynx cirri and trunk tufts

49. Furca shape
    0 - "U"
    1 - "V"

50. Furca composition
    0 - Extend base + adhesive tube
    1 - Short base + adhesive tube
    2 - Extend base + two adhesive tube
    3 - two adhesive tube

51. Base of furca
    0 - 1/3 of furca length
    1 - 2/3 of furca length
    2 - 1/2 of furca length
    3 - >2/3 of furca length

Table 2 - Matrix used in the phylogenetics analysis of Xenotrichulidae

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Chaetonotus mariae	1	0	0	0	0	0	1	1	0	-	-	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
Diuronotus aspetos	0	4	0	0	4	0	1	1	0	-	-	-	2	0	0	4	0	0	1	0	0	-	0	0	0	0
Draculiciteria tessalata	1	0	1	1	0	1	1	1	0	-	-	1	1	0	0	6	0	0	0	0	1	0	1	1	0	0
Xenotrichula (Velox) velox	1	0	0	0	1	1	1	1	1	2	1	2	0	1	1	2	1	0	0	0	1	0	1	0	1	0
Xenotrichula (Velox) cornuta	1	0	0	0	1	1	1	1	1	1	1	2	0	1	1	2	1	0	0	0	1	0	0	0	0	0
Xenotrichula (velox) guadelupensis	1	0	0	0	1	1	1	1	1	1	1	2	0	1	1	2	1	0	1	0	1	0	0	0	0	0
Xenotrichula (Velox) tentaculata	1	1	0	0	0	1	1	1	1	1	1	3	0	0	1	4	0	0	1	1	0	-	0	0	0	0
Xenotrichula sp. nov.	1	0	0	0	1	1	1	1	1	1	1	2	2	1	1	2	1	0	1	0	1	0	0	0	1	0
Xenotrichula (Xenotrichula) intermedia	0	2	0	0	1	1	2	1	0	-	-	2	3	1	0	1	1	0	1	0	1	0	0	0	1	0
Xenotrichula (Xenotrichula) bispina	0	2	0	0	1	1	2	1	0	-	-	2	0	1	0	1	1	0	1	0	1	0	0	0	1	0
Xenotrichula (Xenotrichula) carolinensis	1	3	0	0	1	1	2	1	0	-	-	2	0	1	0	1	1	0	1	0	1	0	0	0	0	0
Xenotrichula (Xenotrichula) floridana	0	2	0	0	1	1	1	1	0	-	-	2	3	0	0	1	1	0	1	0	1	0	0	0	1	1
Xenotrichula (Xenotrichula) laccadivensis	0	1	0	0	0	1	1	1	1	0	1	3	0	0	?	4	0	0	1	0	1	0	0	0	1	1
Xenotrichula (Xenotrichula) lineata	0	2	0	0	3	1	1	1	0	-	-	2	0	0	0	1	1	0	3	0	1	0	0	0	1	0
Xenotrichula (Xenotrichula) paralineata	0	2	0	0	3	1	2	1	0	-	-	2	0	1	0	1	1	0	1	0	1	0	0	0	1	0
Xenotrichula (Xenotrichula) punctata	0	2	0	0	1	1	1	1	0	-	-	2	3	0	0	1	1	0	1	0	1	0	0	0	1	1
Xenotrichula (Xenotrichula) quadritubulata	0	3	0	0	1	1	1	1	0	-	-	2	0	0	0	3	1	1	1	0	1	0	0	0	1	1
Xenotrichula (Xenotrichula) soikai	0	2	0	0	1	1	1	1	0	-	-	2	0	0	0	1	1	0	1	0	0	-	0	0	0	0
Heteroxenotrichula squamosa	0	3	0	0	0	2	1	1	1	3	0	3	0	0	1	5	0	0	2	0	1	1	1	0	1	1
Heteroxenotrichula affinis	0	3	0	0	3	2	1	1	1	0	0	2	0	0	1	2	1	0	2	0	1	1	1	0	1	0
Heteroxenotrichula subterranea	0	3	0	0	3	1	1	1	1	0	0	2	0	0	1	2	1	0	1	0	1	1	1	0	1	0
Heteroxenotrichula wilkeae	0	3	0	0	0	1	1	1	1	0	0	3	0	0	1	5	0	0	2	0	1	1	0	0	1	0
Heteroxenotrichula arcassonensis	0	3	0	0	0	1	1	1	1	0	0	3	0	0	1	5	0	0	1	0	0	-	0	0	0	0
Heteroxenotrichula pygmaea	0	3	0	0	0	1	1	0	1	0	0	3	0	0	1	5	0	0	1	0	1	1	1	0	1	0
Heteroxenotrichula simplex	0	3	0	0	2	2	1	1	1	0	0	2	0	0	1	2	1	0	1	0	1	1	0	0	1	0
Heteroxenotrichula texana	1	0	0	0	2	2	1	0	1	0	0	2	0	0	1	2	1	0	1	0	1	1	0	0	1	0
Heteroxenotrichula transatlantica	0	3	0	0	3	1	1	1	1	0	0	2	0	0	1	2	1	0	1	0	1	1	1	1	0	0

Table 2 – continued

	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Chaetonotus mariae	0	0	0	0	1	0	0	0	0	0	-	0	0	0	0	0	0	0	0	1	0	0	0	1	1	-
Diuronotus aspetos	0	0	0	0	0	0	0	0	0	1	3	1	1	0	0	0	0	0	2	1	0	0	0	1	1	-
Draculiciteria tessalata	0	0	1	0	1	0	0	0	1	1	3	1	1	2	0	0	0	1	0	0	0	0	0	1	0	3
Xenotrichula (Velox) velox	0	0	1	0	1	0	0	0	0	0	-	0	0	2	1	0	0	1	0	0	1	0	0	0	0	1
Xenotrichula (Velox) cornuta	0	0	0	0	0	0	0	0	0	0	-	0	0	2	1	0	0	1	0	0	1	0	0	0	0	1
Xenotrichula (velox) guadelupensis	0	0	0	0	0	0	1	0	0	0	-	0	0	2	1	0	0	1	0	0	1	0	1	0	0	1
Xenotrichula (Velox) tentaculata	0	0	0	0	0	0	0	0	0	0	-	0	0	2	0	0	0	1	0	0	1	0	0	0	0	1
Xenotrichula sp. nov.	1	0	1	0	1	0	1	0	0	1	0	0	0	2	1	0	0	1	0	0	1	0	2	0	0	1
Xenotrichula (Xenotrichula) intermedia	0	0	1	0	1	0	1	1	0	1	1	2	2	2	0	1	0	1	0	0	1	0	0	0	0	0
Xenotrichula (Xenotrichula) bispina	0	0	0	0	1	0	1	0	2	1	2	2	2	1	0	0	0	1	0	0	1	0	0	1	0	1
Xenotrichula (Xenotrichula) carolinensis	0	0	1	1	1	0	1	0	0	1	2	2	2	2	0	1	0	1	0	0	1	0	0	1	0	2
Xenotrichula (Xenotrichula) floridana	0	0	0	0	0	0	0	0	0	1	3	2	2	1	0	1	1	1	0	0	1	0	0	0	0	1
Xenotrichula (Xenotrichula) laccadivensis	0	0	0	0	0	0	0	0	0	0	-	0	0	2	0	0	0	1	0	0	1	1	0	0	0	2
Xenotrichula (Xenotrichula) lineata	0	0	1	1	1	0	1	0	0	0	-	0	0	2	0	1	0	1	0	0	1	0	0	1	0	1
Xenotrichula (Xenotrichula) paralineata	0	0	1	0	1	0	1	0	0	0	-	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1
Xenotrichula (Xenotrichula) punctata	0	0	1	1	0	0	0	0	0	1	4	0	2	1	0	0	0	1	0	0	1	0	0	1	0	1
Xenotrichula (Xenotrichula) quadritubulata	0	0	0	0	1	0	0	0	0	0	-	0	0	2	0	0	0	1	0	0	1	0	0	0	2	1
Xenotrichula (Xenotrichula) soikai	0	0	0	0	0	0	0	0	0	0	-	0	0	1	0	0	0	1	0	0	1	0	0	1	0	2
Heteroxenotrichula squamosa	1	0	1	0	1	0	1	1	0	1	4	1	1	2	0	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula affinis	0	0	1	0	1	0	1	0	0	1	4	0	2	2	1	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula subterranea	0	0	1	0	1	0	1	0	0	0	-	0	0	2	1	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula wilkeae	0	0	1	0	1	0	1	0	0	1	4	0	1	2	1	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula arcassonensis	0	0	0	0	0	0	1	0	0	1	4	1	1	2	0	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula pygmaea	1	0	0	0	1	0	1	1	0	(0/1)	?	?	?	2	(0/1)	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula simplex	0	0	1	0	0	0	1	0	0	1	4	0	2	2	1	0	0	1	1	0	1	0	2	0	0	1
Heteroxenotrichula texana	1	0	0	0	1	0	0	0	0	1	4	2	2	1	0	1	0	1	2	0	1	0	2	0	0	1
Heteroxenotrichula transatlantica	1	0	1	0	1	1	1	0	0	1	4	1	1	2	0	0	0	1	1	0	1	0	2	1	0	1

Results

Under equal weights analysis, four most parsimonious trees (MPTs) with 182 steps, Ci=45 and Ri=62, were obtained (Fig. 5). These four trees recovered the monophyly of the family Xenotrichulidae. The genus *Draculiciteria* is supported by several autapomorphies: conspicuous set of polygonal plates around the large cephalion (character 2[1] and around the well-developed hypostomium (character 3[1]), presence of a plate in the hypostomium region (character 12[1]), unique polygonal-type scales covering all of the dorsal body surface (character 15[6]); *Heteroxenotrichula* is monophyletic and defined by the following synapomorphies: presence of an oval patch of transversal plates (character 4[2]) and by the short tentacles on the lateral head (character 10[0]); only the genus *Xenotrichula* was not recovered as a monophyletic taxon.

Three of the MPT's (Fig. 5A-C) recovered similar topologies: ((*X. tentaculata*, *X. laccadvensis*)(*X. guadelupensis* (*X. cornuta* (*X. velox*(*X.* sp. nov., *Heteroxenotrichula*))))). Two topologies (Figs. 5A-B) present *X. sokai* as sister taxon of *Xenotrichula* species except *X. tentaculata, X. laccadvensis, X. guadelupensis, X. cornuta, X. velox* and *X.* sp. nov. The position of *X. quadritubulata* was recovered differently on these topologies: as a sister taxon of (*X. lineata* (*X. paralineata(X. bispina (X. intermedia, X. carolinenses*)))) (Fig5 A) or as sister taxon of *X. floridana, X. puntacta)* (Fig. 5B). The fourth tree recovered by equal weight analysis (Fig. D) presents a different topology from the previous other three: (*X. tentaculata*, *X. laccadivensis*) was recovered as sister group of all *Xenotrichula* and *Heteroxenotrichula.*

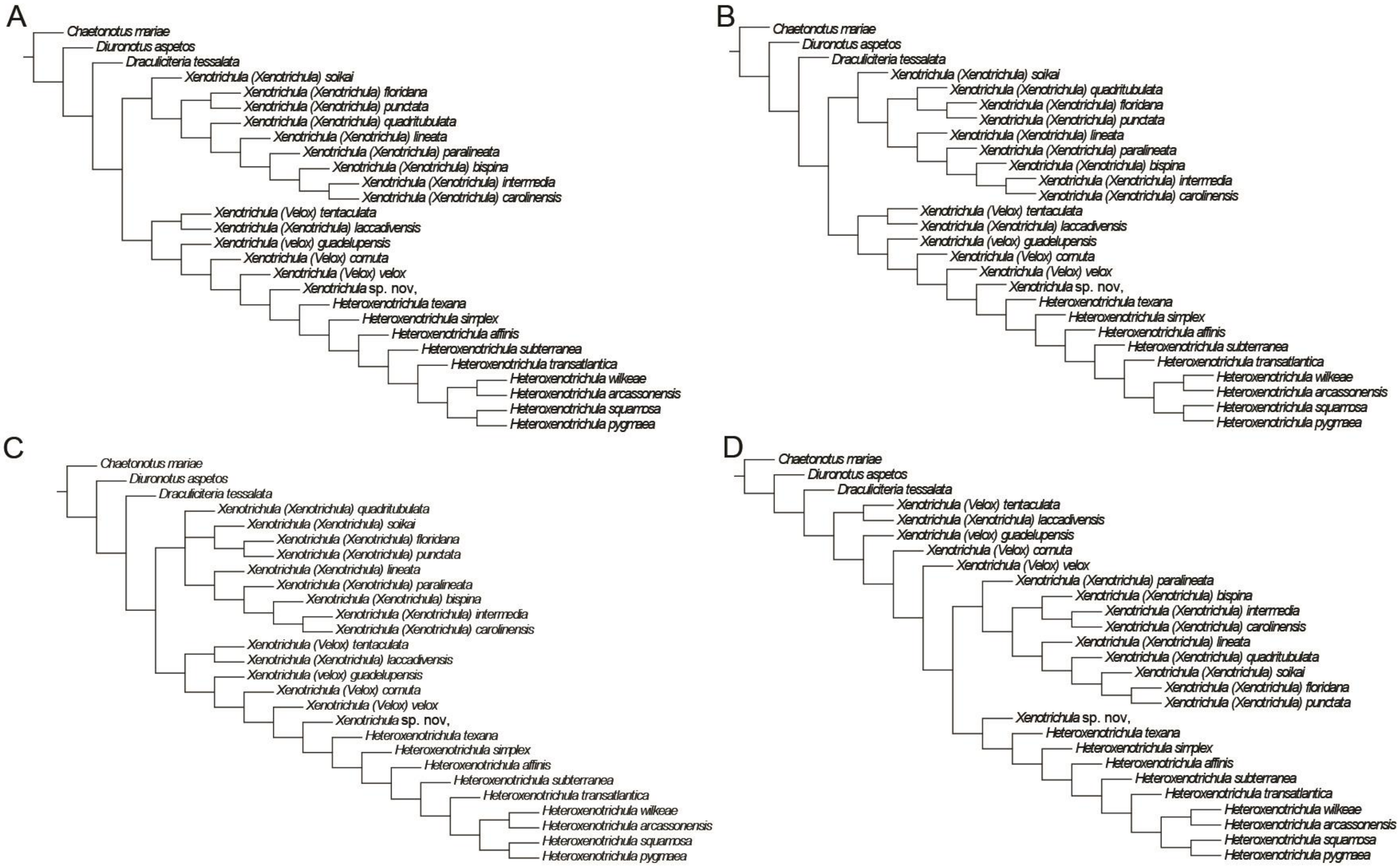

Figure 5 - Four most parsimonious tree founded on equal weight analysis.

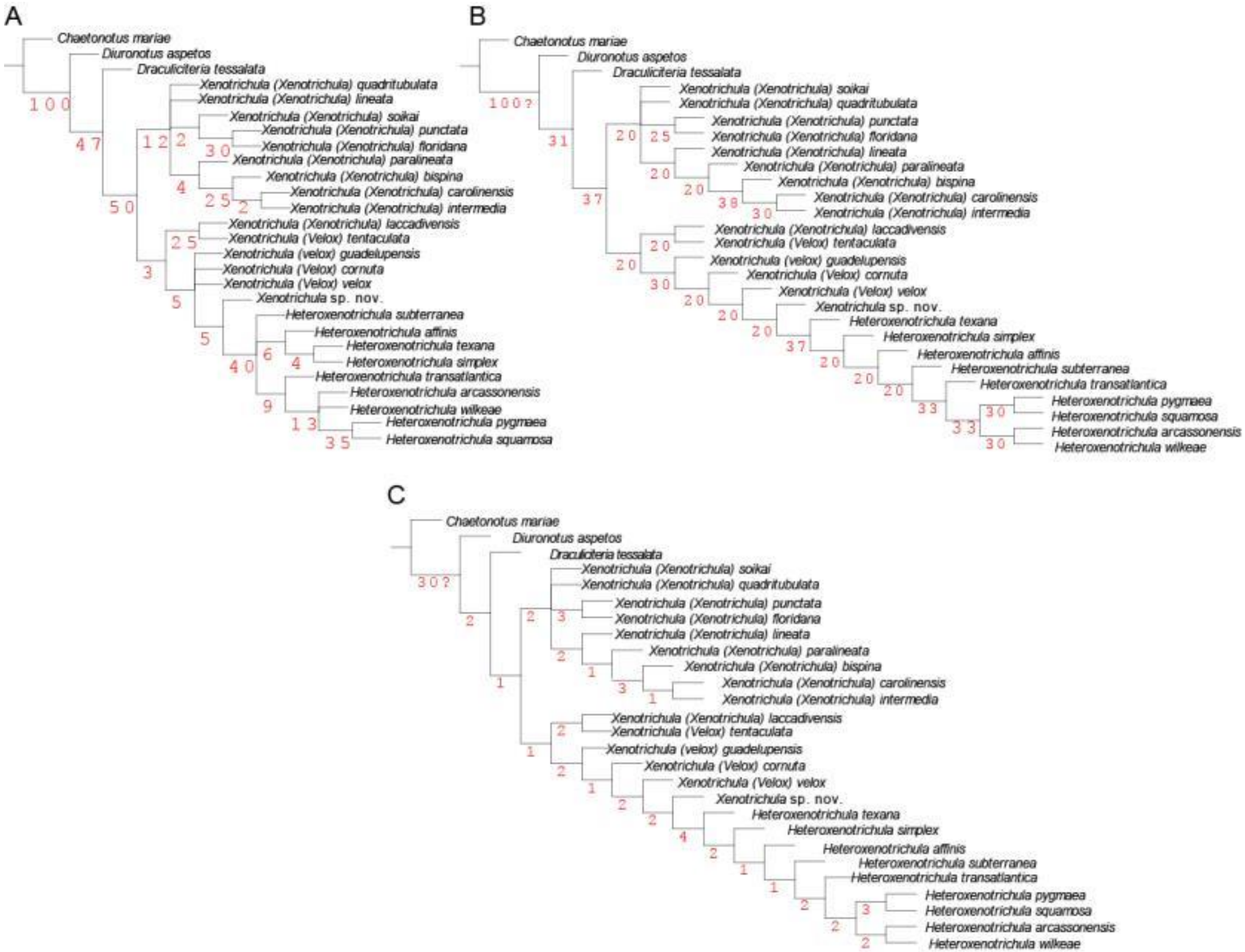

Figure 6 - Branch supports. A. Bootstrap supports. B. Relative Bremer supports. C. Absolute Bremer supports. Numbers are the support values

One MPT was recovered using the implied weight on K-values of 1.193, 1.478, 1.84, 2.315, 2.968, 3.919, 5.434, 8.224, 15.072 and 58.446 and besides that, the present topology was recovered on the unweighted MPT's (Figs. 5B, 7). We prefer this topology, which is used to present the results and discuss the relationships.

The monotypic genus *Draculiciteria,* characterized by the autapomorphyc characters: conspicuous set of poligonals plates around the large cephalion (character 2[1] and around the well-developed hypostomium (character 3[1]), presence of a plate in hypostomium region(character 12[1]), a unique polygonal-type scales covering all the dorsal body (character 15[6]) and by the presence of long spines on the rearmost lateral trunk. This taxon is the sister-group of the clade formed by all other Xenotrichulidae.

The monophyletic taxon formed by all *Xenotrichula* and *Heteroxenotrichula* species is defined by the presence of a pair of locomotory cirri tufts on the middle trunk region (character 46 [1]). Within this major clade are two monophyletic groups. The group formed by (*X. sokai* ((*X. quadritubulata* (*X. floridana*, *X. punctata*)) (*X. lineata* ((*X. paralineata* (*X. bispina* (*X. intermedia, X. carolinensis*))))))) is defined by two synapomorphies: the cephalion region covered by pedunculate scales (character 1[2]) and by the dorsal body region covered by intermedia-type scales (character 15[1]). In this clade we recovered a group who presents a second pair of sensorial bristles on the neck (character 25[1]) formed by (*X. quadritubulata* (*X. floridana, X. punctata*)), a sister group of this were joined by the homoplastic presence of sensorial bristle on the middle of trunk (character 28[1]) and on the caudal appendage (character 32[1]) and is formed by (*X. lineata* ((*X. paralineata* (*X. bispina* (*X. intermedia, X. carolinenses*))))). Along this group the autopomorphic presence of three sensorial cephalic tufts on the head joins (*X. paralineata* (*X. bispina* (*X. intermedia, X. carolinensis*))).

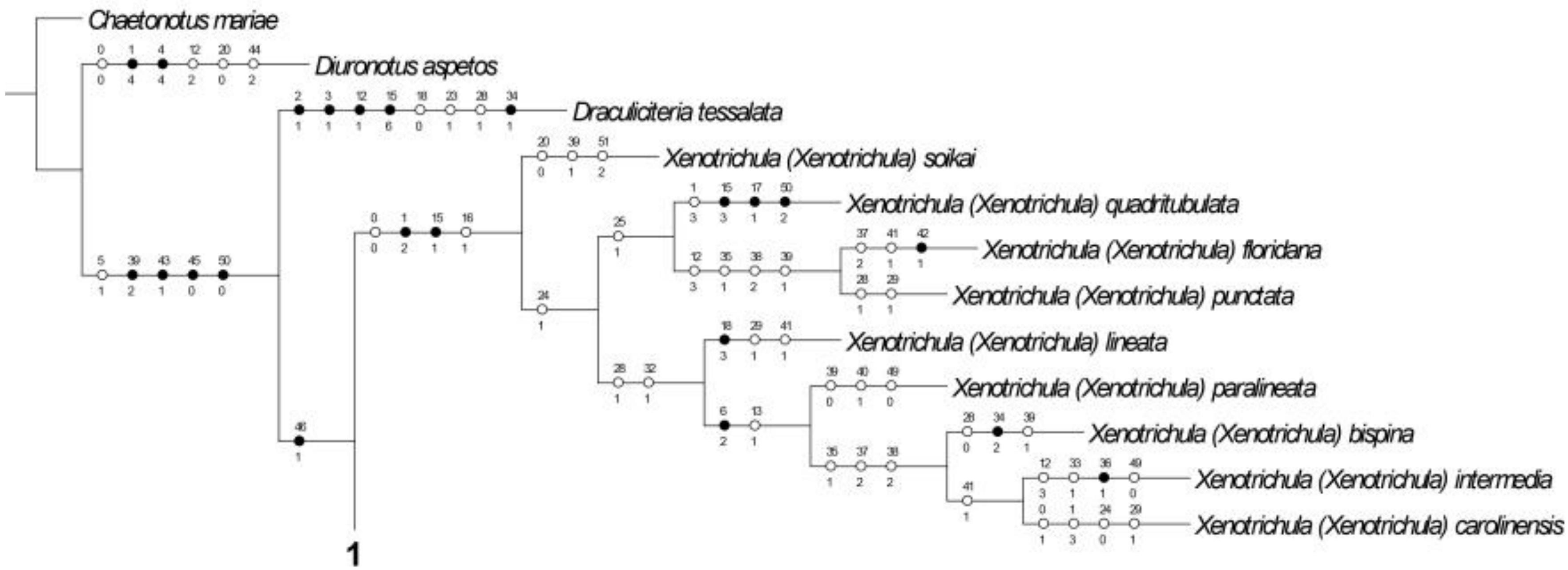

Figure 7 - MPT obtained by implied weight analysis. Black circles = synapomophy; white circles = homoplasy. Numbers above branches are characters; numbers below branches are character states.

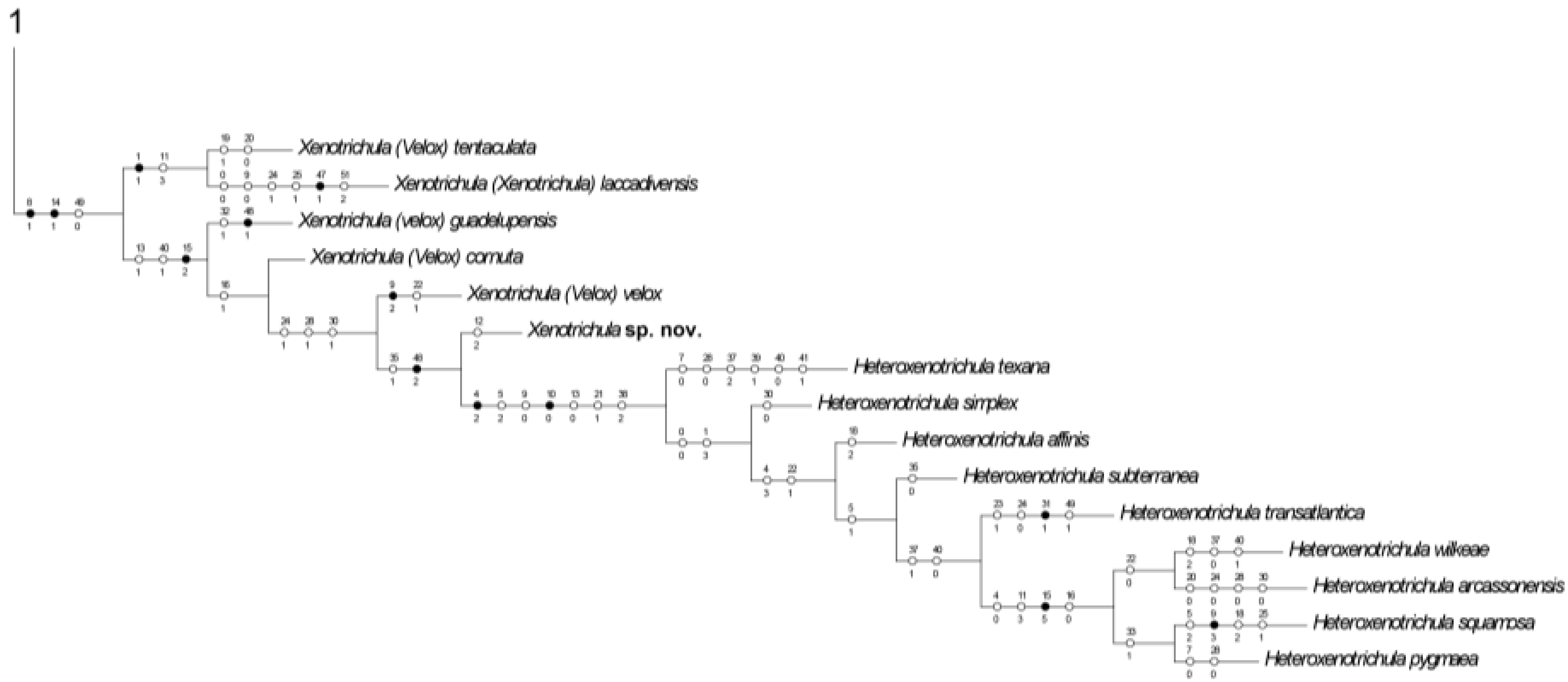

Figure 7 - Continued

The other monophyletic group formed by ((*Xenotrichula tentaculata, Xenotrichula laccadvensis*)(*X. guadelupensis* (*X. cornuta*(*X. velox*(*X.* sp. nov., *Heteroxenotrichula*))))) shares the presence of tentacles (character 8[1]) and the transversal row of cilia from the mouth to the tip of tentacles (character 14[1]). This clade present two monophyletic subclades: (*X. laccadivensis*, *X. tentaculata*) share the presence of small scales covering the cephalion region (character 1[1]) and lateral head (homoplastic character 11[3]), and the presence of elliptic small scales covering the dorsal body (homoplastic character 15[4]). The clade formed by (*X. guadelupensis* (*X. cornuta* (*X. velox* (*X.* sp. nov., *Heteroxenotrichula*)))) shares the autopomorphic subterranea-type scales covering the dorsal body (character 15[2]). Within this clade there are three monophyletic groups: a) (*X.* sp. nov., *Heteroxenotrichula*) shares locomotory cirri of the anterior bands and the trunk cirri with different sizes (character 48[2]); b) *Heteroxenotrichula* shares the presence of an oval patch of transversal plates (character 4[2]) and by the short tentacle on the lateral head (character 10[0]); and c) ((*H. squamosal,H. pygmaea*) (*H. wilkeae, H. arcassonensis*)) share the dorsal body covered by ovals scales (character 15[5]).

Discussion

This study provides the first attempt to understand the phylogenetic relationships among species of Xenotrichulidae using a phylogenetic approach (Hennig, 1966). While Ruppert's (1979) analysis laid the foundation for a classification system based on diagnostic characters, his approach was not phylogenetic because he did not test these characters against an evolutionary hypothesis. In the present study, we take a more contemporary approach by generating phylogenetic hypotheses (cladograms) to examine the distribution of characters, which then provide insights on whether the characters are diagnostic of taxa or not. For this reason, this research can be considered a major contribution to our knowledge of xenotrichulid classification and can be used as the basis for standardizing the terminology associated with xentotrichulid (and Chaetonotida) characters. In addition, this research provides a new summarization of the geographic distribution of all species and a new classification based on this contemporary tree thinking approach.

The first evolutionary scenario for Xenotrichulidae was proposed by Ruppert (1979) based on shared characters and relying solely on his own assumptions about homology. More contemporary approaches to the phylogenetic analysis of Xenotrichulidae have relied on the analysis of morphological characters using a cladistic approach (Kieneke et al. 2008) and molecular data (Paps & Riutort, 2012; Bekkouche & Worsaae, 2016). In Kieneke et al.'s (2008) approach, they used generic characters to propose monophyly for Xenotrichulidae, with *Xenotrichula* as the sister taxon to *Draculiciteria* + *Heteroxenotrichula*. However, as noted in other studies (e.g., Bekkouche and Worsaae 2016), *Xenotrichula* may be an unnatural taxon, so generic characters are not appropriate for phylogenetic analyses until such time that the genus has been demonstrated to be monophyletic. Importantly, as noted below, morphological characters are often ill defined (especially in older literature) and require a phylogenetic approach to determine their homology. In the study by Bekkouche and Worsaae (2016), not only did they recover *Xenotrichula* as non-monophyletic, but they showed *X. velox* to be the sister taxon of *Heteroxenotrichula*; this is identical to the phylogenetic scenario recovered in our own study.

Recognizing some problems with the morphological terminology was the first step toward a better understanding of xenotrichulid phylogeny and classification. Most of xenotrichulid taxonomy is based on cuticular features such as head plates and scales, and many of these have been poorly defined and therefore difficult to homologize and use for systematic purposes. For example, the head plate "hypostomium" is widely used to designate a plate (or set of plates) behind the mouth on the ventral head in many species throughout Chaetonotida, and it is was also used to named two hard projections on each side of mouth in some xenotrichulids. These two structures observed in xenotrichulids are located on different body regions, they are not structurally similar, and thus we can not infer a hypothesis of homology between these projections and the hypostomium of other chaetonotids. Other examples of misinterpretation are seen with the term "cephalion". This structure is usually defined as an anterior plate on the dorsal head region, but some author describes it in Xenotrichulidae as an "undefined area", or an area without scales or plates (just a bare area on the dorsal head)(e.g. Ruppert, 1979; Todaro, 1994). In our current study, we reanalyzed this term and its definitions and generated new hypotheses about the nature of this character: naked, covered by scales, or covered by a plate. We infered that these three arrangements are better defined as individual characters and that the cephalion only has two character states (present or absent).

Other characters have also caused some systematic difficulties but have been clarified in this analysis. Prior to this study, species with long tentacles were grouped with species that lack tentacles, and species with two different scale shapes were grouped into a single clade. Our analyses revealed that all tentacled species share a common ancestry (some *Xenotrichula*, all *Heteroxenotrichula*); prior studies only grouped short-tentacled species of *Heteroxenotrichula* together.

In addition to tentacles and head plates, the shape of the body scales is also a diagnostic character to join species. Previous studies have grouped species by the presence of the subterranea or intermedia-type scales. Now, the subterranea shape is a synapomorphy of all tentaculate species, and the oval shape appears to be a derivative of this more ancestral form and the intermedia shape is a synapomorphy of all untentaculate species.

New proposed classification

Based on the phylogenetic analysis presented above, we propose here a new classification of the family Xenotrichulidae.

The monophyletic family Xenotrichulidae is characterized by the presence of the locomotory cirri. An individual cirrus is composed of a group of cilia that share a common cuticle. The family contains three subfamilies and three genera: Draculiciterinae, genus *Draculiciteria*; Xenotrichulinae, genera *Minutoxenotrichula* gen. nov. and *Xenotrichula* and Rotunduxenotrichulinae subfam. nov., genus *Rotundunxenotrichula* gen. nov.

The subfamily Draculiciterinae remains largely unchanged from Ruppert's (1979) original diagnosis with the only exception being the scale type. Ruppert (1979) described *Draculiciteria tesselata to* possess a compound scale, but with the use of Scanning Electronic Microscopy, we observed single polygonal plates covering the dorsal and ventral body.

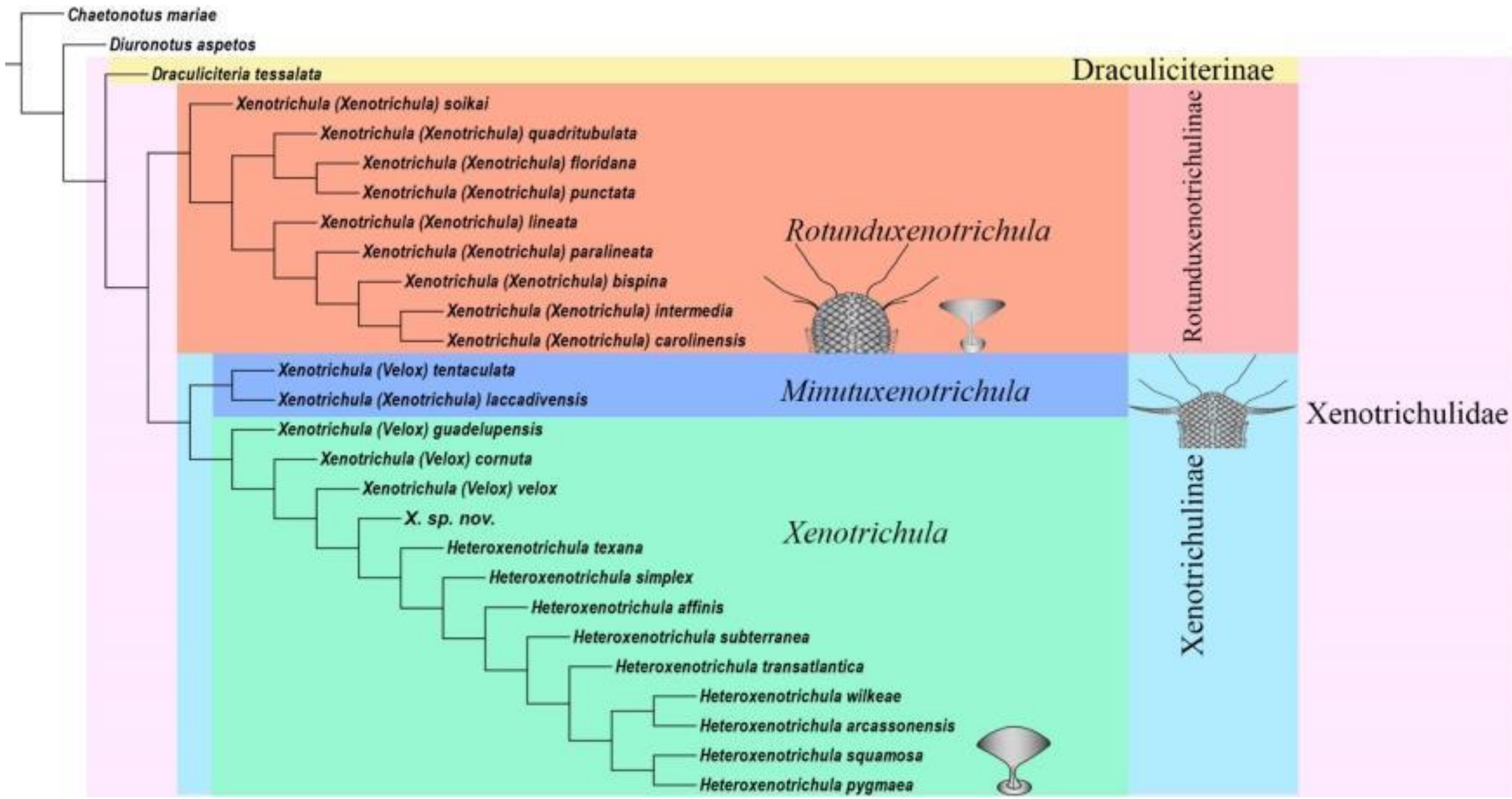

Figure 8 - Phylogenetic tree with new classification proposition. *Rotunduxentrichula* gen. nov. with rounded head and intermedia-type scales, Xenotrichulinae with tentaculated species and *Xenotrichula* with subterranea-type scales.

Ruppert's (1979) classification of Xenotrichulinae originally included species with and without tentacles but he never used scale type as formal subfamily character. The subfamily was divided into *Xenotrichula* and *Heteroxenotrichula*. In this study, we erected a new genus, *Minutuxenotrichula* (see taxonomy section below), which was based on the possession of small elliptical scales (and geographical restriction to India) that differ significantly from the scales of other xenotrichulids. *Minutuxenotrichula* is sister to the genus *Xenotrichula*, which is now a new combination of species (see taxonomy section below). Previously, *Xenotrichula* was composed of species that possess a rounded head or tentacled head; it was distinguished from *Heteroxenotrichula* by tentacle size (short in *Heteroxenotrichula*) and sensorial bristle sizes. Now, the genus *Xenotrichula* contains all species with tentacles (except *Minutuxenotrichula*). The genus *Xenotrichula* is defined by the presence of subterranea-type scales covering the dorsal body wall. The short and naked tentacle is derived from the long scaled tentacles with short cilia on the tips.

The new subfamily Rotunduxenotrichulinae and new genus *Rotunduxenotrichula*, proposed here, is joined by presence of intermedia-type scales covering dorsal body. Regarding the internal relationship just one clade is joined by a synapomorphic character, three sensorial tufts on the head, which is derived from two sensorial tufts on the head. As alluded to before, *R. micracantha* was excluded from this analysis due the lack of morphological characters. Based on the body shape, we think that *R. micracantha* belongs to this genus. However, the real relationship among Rotunduxenotrichulinae remains uncertain.

Taxonomy

Order Chaetonotida Remane, 1925 [Rao and Clausen 1970]

Family Xenotrichulidae Remane, 1927: 294

Subfamily Draculiciterinae Ruppert, 1979: 14

Diagnosis: Xenotrichulidae with elongated body; head with a set of plates in dorsal, ventral and lateral regions; two pairs of sensorial cirri: one pair on dorsal head and a single cirrus on the lateral side of head rising between two lateral plates. Ventral cirri arranged in two lateral longitudinal columns from the head to the middle body (Fig. 9).

Genus *Draculiciteria* Hummon, 1974b: 203

Type-species *Draculiciteria tesselata* (Renaud-Mornant, 1968):142, fig. 3

Emended Diagnosis:

Draculiciterinae with elongated body, head well delimited with a set of plates in dorsal, ventral and lateral regions; curved furca covered 5/6 by flat scales. Ventral cirri organized in two longitudinal columns from the head to the middle trunk. Subterminal mouth followed by a pharynx with anterior bulb. Dorsal and ventral plates/scales are flat with a depression on the posterior edge, the scales shape are not consistent and all shapes are a different type of polygon (Fig. 9).

Draculiciteria tesselata (Renaud-Mornant, 1968):142, fig. 3.

Syn. *Polymerurus tesselatus* Renaud-Mornant, 1968: 142

Diagnosis: Species with a slender body with 270 µm, on average. Anterior mouth width 6 µm in diameter, pharynx 78 µm long. At each laterally rearmost side of body is a single large spine. Well marked head composed dorsally by complex of scales: three pairs of large scales on each side of a large cephalion, one pair of small rectangular scales beside the mouth, a pair of modified epi- and hypopleuria. Dorsal side of body covered by six columns of polygonal scales extending to middle body arising the seventh column at end of the neck extending to top of the furca. Hypostomium as a large plate between eight pairs of scales. Ventral side of body covered by polygonal scales, three columns on the head and five from the neck to end body. Hydrofoil scales present at ventrolateral body. Modified spine at rearmost lateral body. Fourteen flat scales covering inner margin of the adhesive tube, the tip of the tube is naked (average 8 µm long). Two longitudinal bands of locomotory cirri extending from the head to the middle trunk.

Remarks: According to Hochberg and Litvaitis (2001), *Draculiciteria tesselata* presents circular muscles along the pharynx length, longitudinal muscles present along ventral, dorsal and lateral body, heliocoidal muscles are present from the head to half of length of intestine and dorsoventral muscles are present in the lateral and medial positions. Observed under SEM, *Draculiciteria tessalata* possesses single plates (Fig. 9C-E) instead compound scales as proposed by Ruppert (1979).

Marine Realms: Tropical Atlantic, Temperate Northern Atlantic, Temperate South America, Western Indo-Pacific (Fig. 10).

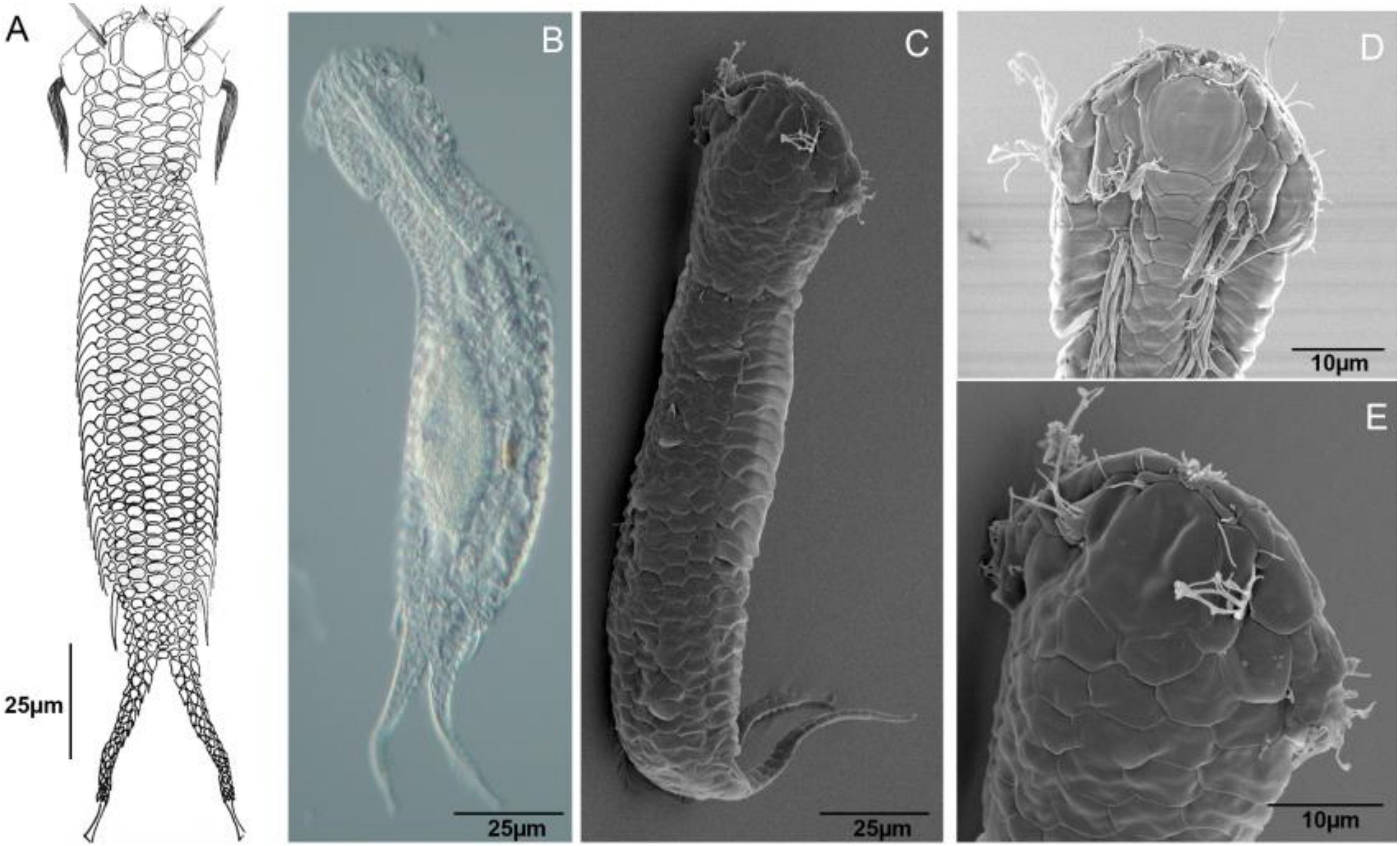

Figure 9 - *Draculiciteria tesselata.* A - Schematic draw of dorsal body (modified from Ruppert (1979); B- DIC microphotography of dorsal body; C- SEM microphotography of dorso-lateral body; D - ventral head; E - Dorsal head.

Table 3 - Geographic coordinates of *Draculiciteria tesselata*

Country	Coordinates	Authors
USA	(42°53'43"N, 70°49'13"W)(18° 21'12"N, 64°46'05"W)(18° 21'12"N, 64°46'05"W)(42°52'39.88"N, 70°48'56.21"W)(43°00'N, 70°40'W)(42°40N, 70°50'W)(25°40'N, 80°10'W)(24°40'N, 81°20'W)(30°14'N, 87°44'W)(27°50'N, 97°03'W)(27°43'N, 97°09'W)(27°05'N, 97°23'W)(30°14'N, 87°44'W)(26°06'N, 97°09'W)(29°12'N, 80°59'W)(25°47'N, 80°07'W)(29°17'N, 80°59'W)(30°01'N, 81°19'W)(29°53'N, 81°17'W)(27°39'N, 80°21'W)(29°55'N, 81°17'W)(27°32'N, 82°43'W)(27°28'N, 82°42'W)(29°13'N, 85°24'W)(26°57'N, 82°23'W)(26°44'N, 82°15'W)(26°43'N, 82°15'W)(28°03'N, 82°49'W)(27°38'N, 82°44'W)(24°39'N, 81°15'W)(25°43'N, 80°09'W)(43°00'N, 70°40'W)(42°42'N, 70°46'W)(42°42'N, 70°47'W)(42°41'N, 70°46'W)(42°50'N, 70°48'W)(43°00'N, 70°40'W)(42°11'N, 70°42'W)(34°41'N, 76°40'W)(34°38'N, 77°05'W)(42°53'N, 70°49'W)(33°20'N, 79°10'W)(33°20'N, 79°10'W)(27°50'N, 97°03'W)(27°43'N, 97°09'W)(27°05'N, 97°23'W)(42°53'N, 70°49'W)(42°53'59.13"N, 70°48'38.12"W)(42°55'58.84"N, 70°47'48.65"W)(43°10'30.90"N, 70°36'30.90"W)(42°52'39.88"N, 70°48'56.21"W)(42°52'39.88"N, 70°48'56.21"W)(42°55'58.84"N, 70°47'48.65"W)	(Hummon, 1974a, 2010; Todaro, Fleeger & Hummon, 1995; Hochberg & Litvaitis, 2001; Kånneby, Todaro & Jondelius, 2012, 2013) Present study
Brazil	(23°21'55.1"S, 44°56'30.2"W)	(Todaro & Rocha, 2005; Hummon, 2010)
British Isles	(49°14'N, 02°12'W)	(Hummon, 2010)
Cyprus	(34°50'N , 32°21'E)(35°02'N , 32°23'E)(34°43'N , 32°25'E)	(Hummon, 2010)
Denmark	(55°05'21.7"N, 8°33'54.5"E)	(Hummon, 2010)
Egypt	(31°,23'N , 27°,00'E)(31°,07'N , 33°,42'E)(31°,08'N , 33°,43'E)(30°,48'N , 29°,03'E)(31°,16'N , 27°,49'E)(31°,17'N , 30°,01'E)(30°,59'N , 28°,42'E)(29°,14'N , 32°,53'E)(27°,56'N , 34°,23'E)	(Hummon, 2010)
England	(52°,58'N, 00°,29'E)	(Hummon, 2010)
France	(43°,39'N , 01°,26'W)(44°,39'N , 01°,15'W)(44°,42'N , 01°,15'W)(44°,44'N , 01°,13'W)(44°,38'N , 01°,13'W)(44°,39'N , 01°,15'W)(41°,42'N , 09°,24'E)(42°,22'N , 09°,31'E)(42°,06'N , 09°,33'E)	(Hummon, 2010)
Germany	(55°02'11.6"N , 8°25'38.6"E)(55°,01'N, 08°,26'E)(54°,54'N, 08°,22'E)(55°,02'N , 08°,26'E)	(Mock, 1979; Hummon, 2010)
Greece	(36°,17'N, 25°,21'E)(39°,08'N, 23°,23'E)(37°,56'N, 24°,01'E)(40°,17'N, 23°,40'E)(40°,05'N, 23°,18'E)(39°,53'N, 22°,51'E)(40°,21'N, 23°,53'E)(35°,19'N, 25°,11'E)(35°,21'N, 24°,19'E)(35°,06'N, 25°,48'E)(35°,11'N, 24°,23'E)	(Hummon, 2010)
Ireland	(53°57'N, 10°,11'W)	(Hummon, 2010)
Isle Of Man	(54°,05'N, 04°,46'W)	(Hummon, 2010)
Israel	(33°,02'N, 35°,05'E)(31°,40'N, 34°,34'E)(32°,43'N, 34°,56'E)(31°,53'N, 34°,41'E)(32°,04'N, 34°,45'E)(29°,32'N, 34°,57'E)(29°,30'N, 34°,55'E)	(Hummon, 2010)
Italy	(40°49'54.3"N , 14°14'10.4"E)(42°45'01.8"N , 10°21'33.8"E)(43°03'00.0"N , 9°50'07.1"E)(43°01'36.4"N , 9°50'29.1"E)(38°,58'N, 08°,26'E)(39°,08'N, 09°,33'E)(40°,37'N, 09°,46'E)(40°,26'N, 09°,50'E)(43°,01'N, 09°,49'E)(43°,01'N, 09°,49'E)(43°40'26.8"N , 10°16'16.2"E)(43°40'46.4"N , 10°16'03.5"E)(40°,54'N, 12°,57'E)(40°47'N, 09°41'E)(42°39'N, 11°04'E)(45°,21'N, 12°,19'E)(45°,37'N, 13°,02'E)(42°,07'N, 15°,30'E)(45°,32'N , 12°,45'E)(41°,54'N, 15°,21'E)(40°,43'N, 17°,48'E)(45°,40'N, 13°,07'E)(43°,25'N , 13°,36'E)(41°,51'N, 16°,11'E)(43°,58'N, 12°,55'E)(39°,49'N, 18°,15'E)(40°,25'N, 16°,49'E)(39°,50'N, 18°,13'E)(39°,48'N, 18°,22'E)(43°,09'N, 10°,40'E)(43°,55'N, 10°,13'E)(43°,01'N, 09°,49'E)(44°,25'N, 08°,56'E)(43°,44'N , 10°,16'E)(43°,04'N, 09°,50'E)(43°,44'N, 10°,16'E)(35°,30'N, 12°,37'E)(38°,58'N, 08°,26'E)(42°,49'N, 10°,44'E)(42°,39'N, 11°,04'E)(40°,37'N, 09°,46'E)(40°,00'N, 15°,21'E)(41°,47'N, 12°,13'E)(40°,44'N, 13°,49'E)(42°,27'N, 11°,09'E)(40°,26'N, 09°,50'E)(40°,54'N, 12°,57'E)(42°,20'N, 11°,33'E)(42°,02'N, 11°,56'E)(41°,12'N, 13°,33'E)(39°,08'N, 09°,33'E)(41°,16'N, 13°,25'E)(42°,14'N, 11°,42'E)	(Renaud-Mornant, 1968; Luporini, Magagnini & Tongiorgi, 1971; Balsamo, Todaro & Tongiorgi, 1992; Todaro, 1992; Balsamo, Fregni & Tongiorgi, 1995; Todaro et al., 2003; Hummon, 2010; Kånneby, Todaro & Jondelius, 2012, 2013)
Kuwait	(29°30'N; 48°01'E)	(Leasi & Todaro, 2008)
Portugal	(37°49'N, 25°24'W)(38°35'N, 28°40'W)	(Hummon, 2010)
Scotland	(58°,04'N, 05°,18'W)(58°,34'N, 04°,43'W)(57°,49'N, 05°,41'W)(58°,30'N, 04°,22'W)	(Hummon, 2010)
Virgin Islands	(18°20'N, 64°50'W)(18°,21'N, 64°,52'W)	(Hummon, 2010)
Wales	(52°,28'N, 03°,56'W)(52°,56'N, 04°,39'W)	(Hummon, 2010)

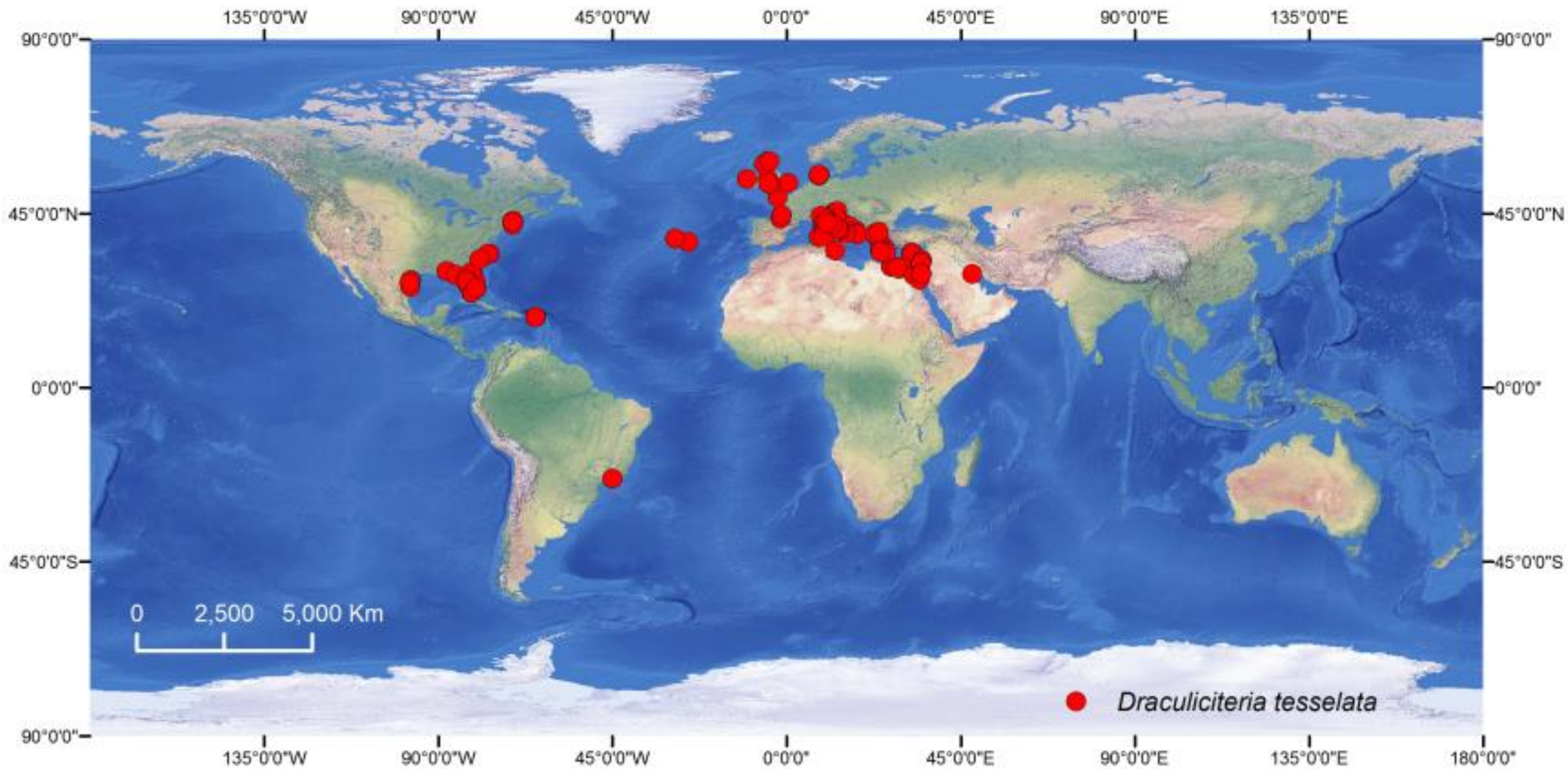

Figure 10 - Map of distribution of *D. tesselata*

Subfamily Xenotrichulinae Remane, 1927
Type Genus *Xenotrichula* **(Remane, 1927)**: 289

Emended

Xenotrichulidae with somewhat short body, semicircular in cross section, head weakly delimited and furca weakly delimited from the trunk. Head presenting long or short tentacles which can possess scales and/or cilia. Dorsal head region present as naked cuticle, with scales, or singular cephalion. An oval patch of transversal flat plates can be present on the central dorsal head. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle ("Kammantiger Zacken"). Short pharynx. Dorsal body covered by elliptical or subterranea-type or flat oval scales. Furca bases covered by flat scales. Hydrofoil scales usually present on the ventral lateral body. Ventrally the body can be covered by scales and can present longitudinal plates on the middle trunk to the end. Ventral locomotory cirri in different sizes anteriorly, the first portion of head can be all covered by cirri and divided in two longitudinal bands to the PHJIn, a pair of locomotory cirri on the middle trunk. Testis with caudal anastomosis. X-organ present.

Genus *Xenotrichula* Remane, 1927**:** 289
Type-species *Xenotrichula velox* Remane, 1927: 289, figs. 1-3

Emended Diagnosis:

Xenotrichulinae with head presenting long or short tentacle which can possess scales and/or cilia. Cephalion can be present as a plate or the region can be naked or covered by scales. An oval patch of transversal flat plates can be present on the dorsal head. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle ("Kammantiger Zacken"). Short pharynx. Dorsal body covered by subterranea-type or flat oval scales. Hydrofoil scales usually present on the ventrolateral body. Ventral side of body covered by scales and can present longitudinal plates on the middle trunk to the end. Furca bases covered by flat scales. Ventral locomotory cirri of different sizes anteriorly, the first portion of head can be all covered by cirri and divided in two longitudinal bands to the PHJIn, a pair of testis with caudal anastomosis. X-organ present.

Xenotrichula velox Remane, 1927: 289, figs. 1-3

Species with body 275 µm long, pharynx length 60 µm. Head with a pair of long lateral tentacles. Cephalion as small plate. Two pairs of dorsal and lateral sensorial cirri. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Two hard projections on each side of mouth (previously referred to as Bidentate hypostomium). Dorsal body covered by 14 to 18 rows of pedunculated subterranea-type scales extending to the top of furca. Hydrofoil scales present on the ventrolateral body. Furcal base is covered by eleven flat scales in the inner margin. Four rows of plate at posterior part of the trunk, inners rows present 20 plates. Two longitudinal bands of ventral locomotory cirri increasing in size along the pharynx length (5 µm to 25 µm). One pair of locomotory cirri tufts at middle trunk (10 µm) (Fig. 11 A-B).

Marine Realms: Temperate Northern Atlantic, Western Indo-Pacific, Artic (Fig. 12).

Table 4 - Geographic coordinates of *Xenotrichula velox*

Country	Coordinates	Authors
Algeria	(35°42'N, 00°52'W); (36°36'N, 02°26'E); (35°43'N, 00°51'W); (35°34'N, 01°09'W); (35°39'N, 01°03'W); (35°44'N, 00°50'W); (35°18'N, 01°21'W); (35°45'N, 00°48'W); (36°12'N, 00°26'E); (35°06'N, 01°50'W); (35°25'N, 01°14'W); (36°42'N, 02°49'E);	(Hummon, 2010)
Belgium	(51°04'24.4"N, 3°40'50.5"E); (51°13'N, 02°53'E);	(Jouk et al., 1992; Hummon, 2010)
British Isles	(49°10'N, 02°11'W);	Hummon 2010
Denmark	(55°05'21.7"N,08°33'54.5"E); (55°04'N	(Mock, 1979; Hummon, 2010)
England	(49°56'N, 06°18'W); (49°58'N, 06°17'W); (49°57'N, 06°16'W); (49°56'N, 06°19'W); (52°58'N, 00°29'E); (52°56'N, 00°19'E); (49°58'N, 06°17'W); (52°27'N, 01°31'E); (49°54'N, 06°18'W); (55°01'N, 01°26'W); (50°36'N, 03°24'W); (50°23'N, 03°32'W); (49°53'N, 06°21'W);	(Hummon & Warwick, 1990; Hummon, 2010)
France	(44°44'30.5"N, 1°06'15.5"W); (48°43'24.9"N , 3°59'31.9"W); (48°43'37.6"N, 3°59'15.5"W); (49°40'06.1"N , 1°29'18.3"W); (48°41'17.1"N , 3°55'33.2"W); (48°38'27.9"N , 4°18'42.8"W); (43°16'48.8"N , 5°20'59.8"E); (44°38'N, 01°13'W); (48°43'N, 03°59'W); (50°46'N, 01°35'E); (44°39'N, 01°15'W); (44°45'N, 01°09'W); (48°42'N, 04°00'W); (44°38'N, 01°15'W); (44°38'N, 01°07'W); (50°48'N, 01°35'E); (44°43'N, 01°06'W); (44°38'N, 01°14'W); (48°40'N, 03°56'W); (44°39'N, 01°15'W); (44°39'N, 01°12'W); (44°44'N, 01°13'W); (44°39'N, 01°10'W); (43°31'N, 03°57'E); (43°11'N, 05°41'E);	(Swedmark, 1956; D'Hondt, 1966, 1970; Kisielewski, 1988; Hummon, 2010)
Germany	(54°22'53.5"N , 10°58'39.2"E); (55°02'11.6"N , 8°25'38.6"E); (54°54'47.8"N , 8°21'51.4"E); (54°48'00.6"N , 8°17'45.6"E); (54°46'N, 10°14'E); (54°22'N, 10°59'E); (54°46'N, 10°14'E); (54°18'N, 10°46'E); (55°01'N, 08°26'E); (54°54'N, 08°22'E); (54°48'N, 08°18'E); (55°02'N , 08°26'E);	(Remane, 1927; Forneris, 1961; Mock, 1979; Ruppert, 1979; Hummon, 2010)
Grecee	(39°56'N, 22°42'E);	(Hummon, 2010)
India	(17°42'N, 83°18'E); (17°44'N, 83°20'E); (17°41'N, 83°17'E); (17°43'N, 83°20'E); (17°42'N, 83°19'E); (17°32'55.2"N, 83°11'06.8"E); (11°29'24.0"N , 92°43'41.9"E); (10°33'42.5"N , 72°37'55.0"E); (19°33'12.2"N , 85°18'26.0"E); (19°52'N, 86°07'E); (19°51'N, 86°03'E); (19°46'N, 85°46'E); (19°47'N, 85°49'E); (19°52'N, 86°07'E); (19°51'N, 86°03'E); (19°46'N, 85°46'E); (19°47'N, 85°49'E); (17°32'55.2"N, 83°11'06.8"E); (10°33'N, 72°38'E); (10°49'N, 73°41'E); (11°13'N , 72°47'E); (19°15'N, 84°55'E); (17°42'N, 83°18'E); (10°33'N, 72°38'E); (19°51'N, 86°03'E); (19°52'N, 86°07'E); (17°44'N, 83°20'E); (17°41'N, 83°17'E); (17°43'N, 83°20'E); (19°46'N, 85°46'E); (19°47'N, 85°49'E); (19°48'N, 85°51'E); (17°42'N, 83°19'E);	(Rao & Ganapati, 1967; Rao, 1969, 1989, 1991; Naidu & Rao, 2004; Hummon, 2010)
Isle Of Man	(54°06'N, 04°39'W); (54°05'N , 04°46'W); (54°17'N, 04°35'W);	(Hummon, 2010)
Poland	(53°56'48.7"N , 14°28'20.6"E); (54°55'N, 18°21'E); (54°43'N, 17°12'E); (54°44'57.7"N , 18°40'09.7"E); (53°55' N, 14°26' E); (54°40' N, 17°02' E); (54°43' N, 17°13' E); (54°45' N, 18°30' E); (54°44' N, 18°34' E); (54°36'31" N, 18°49'65" E); (54°36'22" N, 18°47'58" E); (54°43' N, 18°34' E); (54°55'N, 18°21'E); (54°43'N, 17°12'E); (54°44'57.7"N , 18°40'09.7"E);	(Kisielewski, 1975; Hummon, 2008, 2010; KOLICKA et al., 2014).
Portugal	(37°01'N, 08°57'W);	(Hummon, 2010)
Russia	(66°20'N, 33°40'E);	(Hummon, 2010)
Scotland	(55°41'43.9"N , 4°53'57.7"W); (58°10'N, 05°14'W); (58°31'N, 04°12'W); (54°58'N, 05°04'W); (56°56'N, 05°51'W); (56°37'N, 02°30'W); (56°41'N, 02°26'W); (55°18'N, 04°50'W); (58°30'N, 04°22'W); (57°20'N, 01°56'W); (57°24'N, 01°51'W); (58°34'N, 04°43'W); (55°37'N, 04°44'W); (56°28'N, 02°49'W); (55°56'N, 03°06'W); (57°34'N, 01°50'W); (56°23'N, 02°48'W);	(Hummon, 1975, 2010)
Sweden	(57°38'10.6"N , 18°16'54.5"E); (55º54'22"N, 14º17'41"); (55º54'22"N, 14º17'41"); (57°29'N, 18°05'E); (56°37'N, 12°54'E); (56°37'N, 12°54'E); (57°29'N, 18°05'E);	(Karling, 1954; Jansson, 1968; Hummon, 2010; Kånneby, Todaro & Jondelius, 2012, 2013)
Wales	(52°51'N, 04°34'W); (53°14'N, 04°03'W); (53°16'N, 04°42'W); (53°17'N, 04°07'W); (52°51'N, 04°34'W); (53°14'N, 04°03'W); (53°16'N, 04°42'W); (52°56'N, 04°39'W);	(Boaden, 1963; Hummon, 2010)

Xenotrichula guadelupensis Kisielewski, 1984: 37, figs. 1-3

Species with body 170-180 µm long, pharynx length 46-49 µm. Head with a pair of long lateral tentacles (21-24 µm) covered by rhomboid-like scales and bearing short cilia at end. Cephalion as small plate. Two pairs of dorsal and lateral sensorial cirri. Dorsal body covered by 13 rows of pedunculated subterranea-type scales extending to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly. Furcal base covered by flat scales. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Six longitudinal rows of flat large plates are present at ventral trunk side, inners rows presents 20 plates. . Two longitudinal bands locomotory cirri increase in size along the pharynx length (5 µm to 11 µm). One pair of locomotory cirri tufts at middle trunk (10 µm) (Fig, 11C).

Marine Realms: Tropical Atlantic, Tropical Eastern Pacific (Fig. 12)

Table 5 - Geographic coordinates of *Xenotrichula guadelupensis*

Country	Coordinates	Authors
Guadeloupe	(16°03'N, 61°45,4' W); (15°57,8' N, 61°40'W)	(Kisielewski, 1984; Hummon, 2010)
Ecuador	(01°33'S, 80°48'W)	(Hummon, 2010)

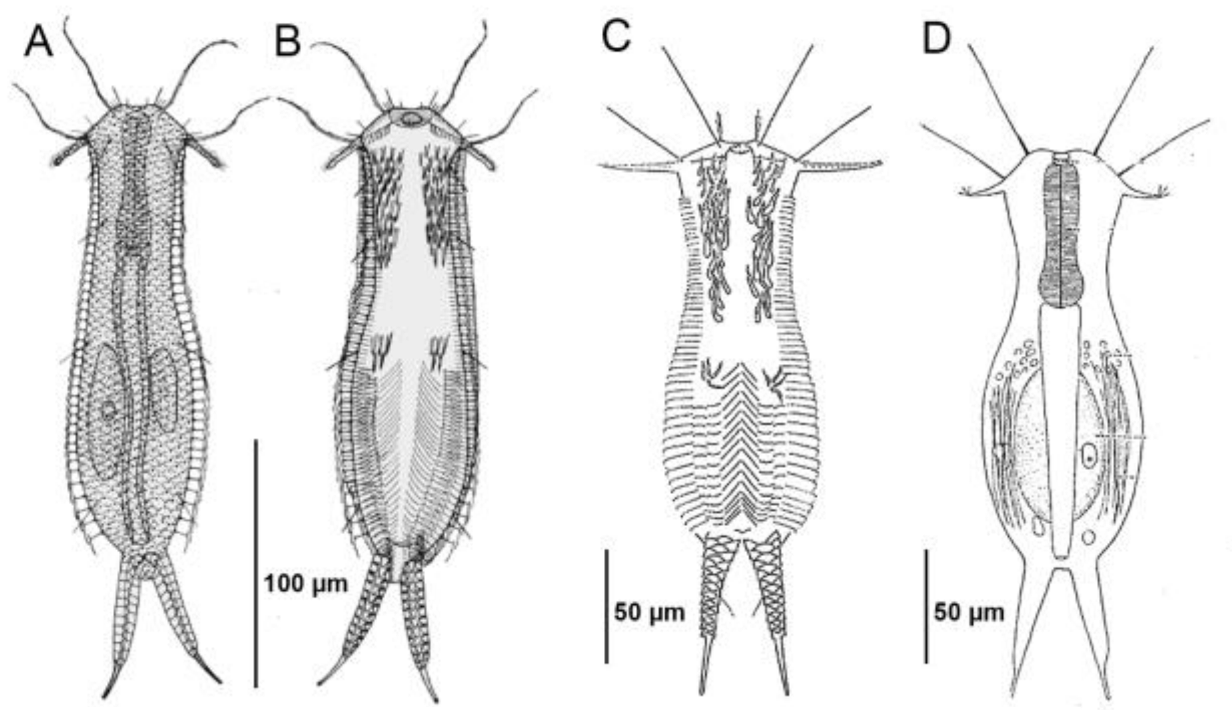

Figure 2 - A-B, *X. velox* (Modifyed from Hummon 2008)*:* A- Dorsal body; B- Ventral body. C- Ventral body of *X. guadelupenses.* D - Ventral body of *X. cornuta*

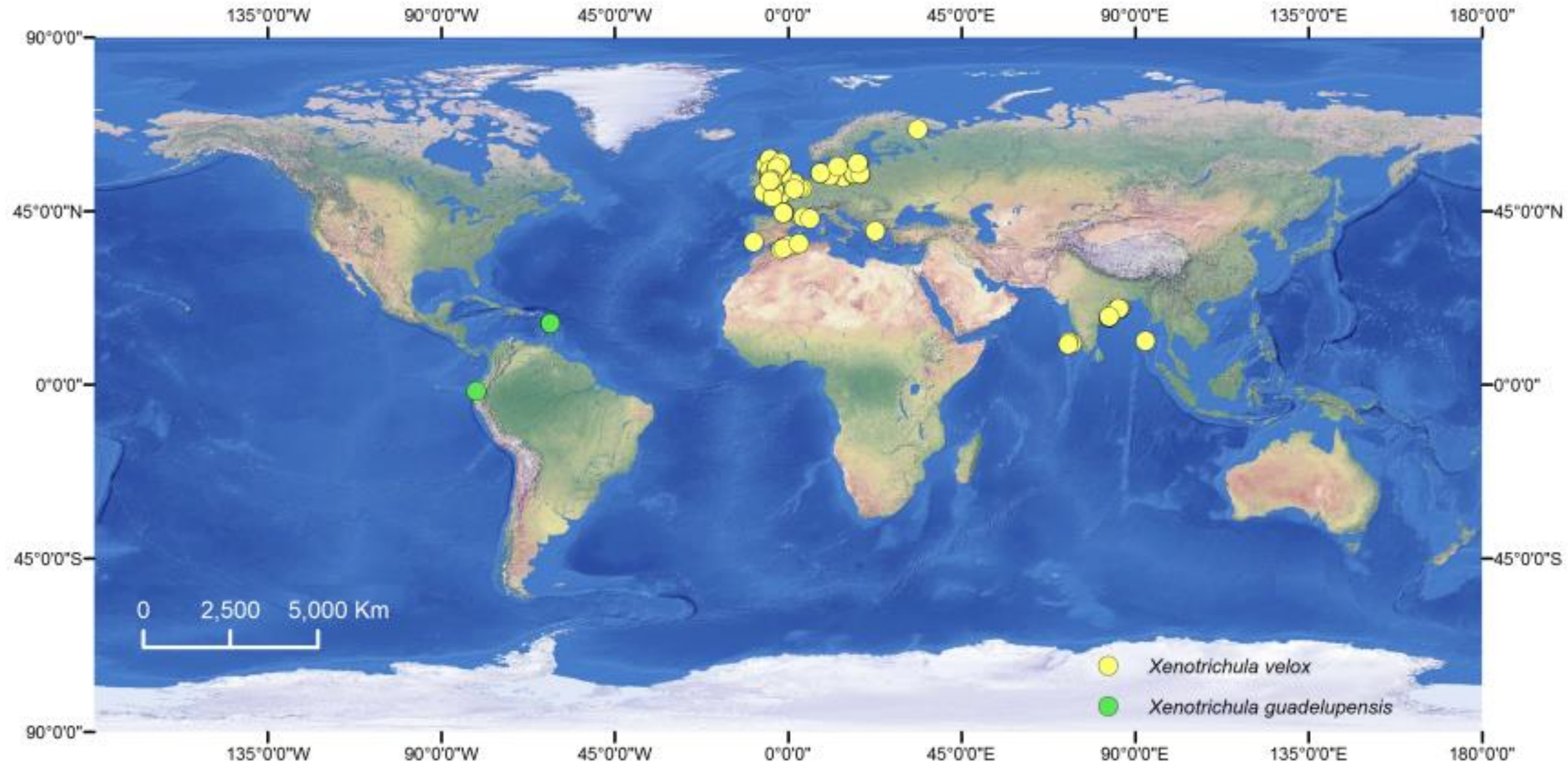

Figure 12 - Map of distribution of *X. velox* and *X. guadelupenses*

Xenotrichula cornuta Wilke, 1954: 520, figs. 15-16

Species with body up 250 µm long, pharynx length 70µm. Head with a pair of long lateral tentacles (20 µm) covered by scales and bearing short cilia at end. Two pairs of antero-dorsal sensorial cirri. Cephalion as small plate. Two hard projections on each side of mouth (old Bidentate hypostomium). Dorsal body covered by 16 rows of pedunculated subterranea-type scales extending to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly. Furcal base covered by flat scales. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Four longitudinal rows of flat scales are present in each side of middle central at ventral trunk side. Two longitudinal bands locomotory cirri along the pharynx length. One pair of locomotory cirri tufts at middle trunk (Fig. 11D).

Marine Realms: Temperate Northern Atlantic (Fig. 13).

Table 6 - Geographic coordinates of *Xenotrichula cornuta*

Country	Coordinates	Authors
Italy	(40°24'48.5"N , 14°59'19.5"E); (39°52’N, 15°46’E); (40°25’N, 14°58’E); (39°52’N, 15°46’E);	(Wilke, 1954; Hummon, 2010)
France	(50°59'49.4"N , 2°01'45.0"E); (43°39'48.5"N , 1°25'52.9"W); (45°41'47.8"N , 1°12'26.5"W); (50°26'N, 01°33'E); (45°41'N, 01°13'W); (43°39'N, 01°26'W); (44°39'N, 01°15'W); (51°00'N, 02°04'E); (51°03'N, 02°23'E); (50°53'N, 01°38'E); (45°47'N, 01°13'W);	(Hummon, 2010)

Xenotrichula sp. nov.

Species with body up 150 µm; pharynx length to 41.5µm. Head with a pair of long lateral scaled tentacles (22.5 µm) covered by scales and bearing short cilia on the tips. Dorsal body covered by 17 rows of pedunculated subterranea-type scales extending to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly. Furcal base covered by flat scales. Ventral body covered by double edge scales along the pharynx length. Two longitudinal rows of 21 flat scales are present in each side of middle central at ventral trunk side, from the middle trunk to the top furca. Two longitudinal bands locomotory cirri along the pharynx length. One pair of locomotory cirri tufts at middle trunk.

Marine Realms: Tropical Atlantic, Temperate South America (Fig. 13).

Table 7 - Geographic coordinates of *Xenotrichula* sp. nov.

Country	Coordinates	Authors

Brazil	(17º54' S, 39º22' W); (23°38'S, 45°25'W)	Present Study

Xenotrichula texana (Todaro, 1994) **nov. comb.**

Heteroxenotrichula texana Todaro, 1994:18, figs. 3-4

Species with stumpy body 105 µm long, subterminal mouth, pharynx length 32 µm. Head possess small lateral tentacles. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Two pairs of antero-dorsal sensorial cirri. Cephalion as small plate. Oval patch of 12-13 flat plates on the central area of the head. Dorsal body covered by four rows of pedunculated subterranea-type scales on each side of oval patch and 17 rows of subterranea-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Ventral body covered by pedunculated subterranea-type scales from the U48 to to the top of furca. Locomotory cirri covering the pharynx length of ventral side, different size of cirri (7-10 µm) and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk (Fig. 14A).

Marine Realms: Temperate Northern Atlantic (Fig. 13).

Table 8 - Geographic coordinates of *Xenotrichula texana*

Country	Coordinates	Authors
USA	(27°28'N, 97°16'W);(29°11'N, 94°57'W);(27°28'N, 97°16'W);(29°11'N, 94°57'W);	(Todaro, 1994; Hummon, 2010)

Xenotrichula simplex Mock, 1979 **stat. rev.**

Syn. *Heteroxenotrichula simplex* (Mock, 1979):423, fig. 10

Species with stumpy body 160 µm long, subterminal mouth, pharynx length 37 µm. Head possess small lateral tentacles. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Oval patch of 12-13 flat plates on the central area of the head. Dorsal body covered by three rows of pedunculated subterranea-type scales on each side of oval patch and 18 rows of subterranea-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Ventral body covered by pedunculated subterranea-type scales from the U48 to the top of furca. Two longitudinal rows of flat scales are present in each side of middle central at ventral trunk side. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk (Fig. 14C).

Marine Realms: Temperate Northern Atlantic (Fig. 13).

Table 9 - Geographic coordinates of *Xenotrichula simplex*

Country	Coordinates	Authors
Germany	(55°02'11.6"N , 8°25'38.6"E);(55°03'27.4"N , 8°25'25.4"E);(55°00'N, 08°23'E);(55°02'N , 08°26'E);	(Mock, 1979; Hummon, 2010)

Xenotrichula transatlantica (Ruppert, 1979) **nov. comb.**

Heteroxenotrichula transatlantica Ruppert, 1979: 269, figs. 15-17

Species with body up to 172 µm long, subterminal mouth, pharynx length 31 µm. Head with a pair of small lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Oval patch of 27 flat plates on the central area of the head. Dorsal body covered by three rows of pedunculated subterranea-type scales on each side of oval patch and 16 rows of subterranea-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Furcal base covered by flat scales. Ventral body covered by six rows of pedunculate scales from the U48 to the top of furca. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 14B).

Marine Realms: Temperate Northern Atlantic (Fig. 13).

Table 10 - Geographic coordinates of *Xenotrichula transatlantica*

Country	Coordinates	Authors
USA	(34°38'30"N, 77°05'23"W)	(Ruppert, 1979; Hummon, 2010)

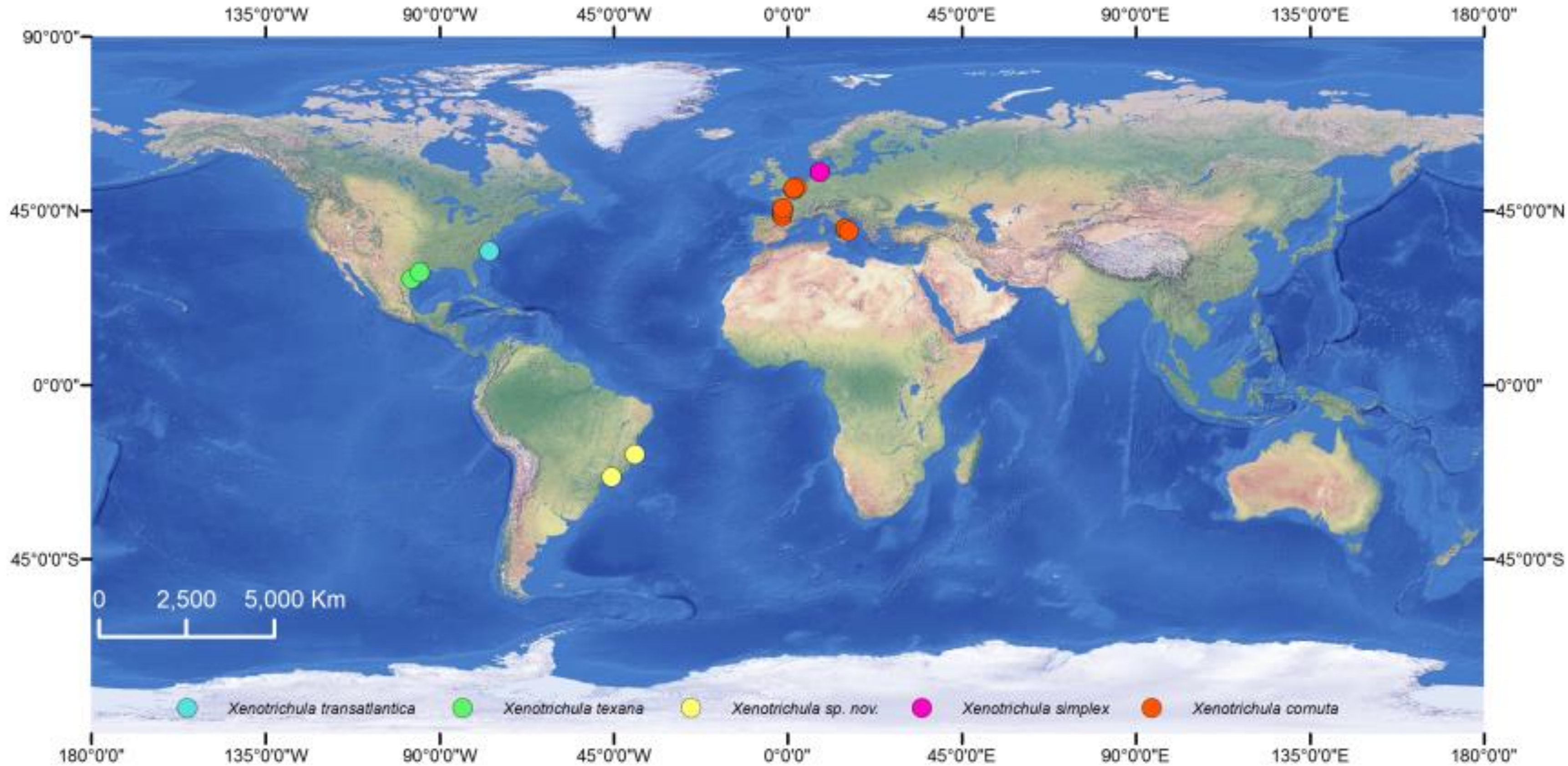

Figure 13 - Map of distribution of *X. transatlantica, X. texana, X.* sp. nov., *X. simplex* and *X. cornuta.*

Xenotrichula wilkeae (Ruppert, 1979): 41, figs. 21-22 **nov. comb.**

Heteroxenotrichula wilkeae Ruppert, 1979: 41

Species with body up to 220 µm, subterminal mouth, pharynx length 41.5 µm. Head with a pair of small lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Dorsal body covered by nine rows of oval scales increasing in size anterior-posteriorly to the top of furca. Furcal base covered by flat scales. Ventral body covered two rows of oval scales on each lateral ventral. Four rows of plate at posterior part of the trunk, inners rows present 20 plates. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 14D).

Remarks: By the same reasons as pointed by Hummon and Todaro (2010) we rejected the synonymizing of *H. variocirrata* with *H. wilkeae* as stated by d'Hondt & Balsamo (2009).

Marine Realms: Temperate Northern Atlantic (Fig. 16).

Table 11 - Geographic coordinates of *Xenotrichula wilkeae*

Country	Coordinates	Authors
France	(44°35'42"N, 01°35'09"W); (44°40'00"N, 01°30'06"W); (44°39'N, 01°10'W); (44°37'N, 01°17'W)	(Ruppert, 1979; Hummon, 2010)
Germany	(55°02'11.6"N,08°25'38.6"E); (55°,01'N, 08°,26'E)	(Mock, 1979; Hummon, 2010)
Portugal	(37°01'N, 08°57'W)	(Hummon, 2010)

Xenotrichula affinis Remane, 1934 **stat. rev.**

Heteroxenotrichula affinis (Remane, 1934): 477, fig. 4

Syn. *Xenotrichula variocirrata* d'Hondt, 1966b:7

Species with body up to 141 µm long, subterminal mouth, pharynx length 35 µm. Head with a pair of small lateral tentacles. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Oval patch of 15 flat plates on the central area of the head. Dorsal body covered by three rows of pedunculated subterranea-type scales on each side of oval patch and 16 rows of subterranea-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Ventral body covered five rows of subterranea-type scales on each lateral body from U51 to the top of furca. Three rows of plate at midventrally posterior part of the trunk. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided

(U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 14E).

Marine Realms: Temperate Northern Atlantic, Central Indo-Pacific (Fig.16).

Remarks. As pointed by Hummon and Todaro (2010), the new morphological information provide by D`Hondt and Balsamo (2009) need to be confirmed to justify the synonimization with *X. affinis.* The morphological description of the specimens analyzed was based in old microphotographs, not based on live animals, presents discrepancies between scales shape and distribution, we agree with Hummon and Todaro (2010) in consider this species as *Species inquirenda.*

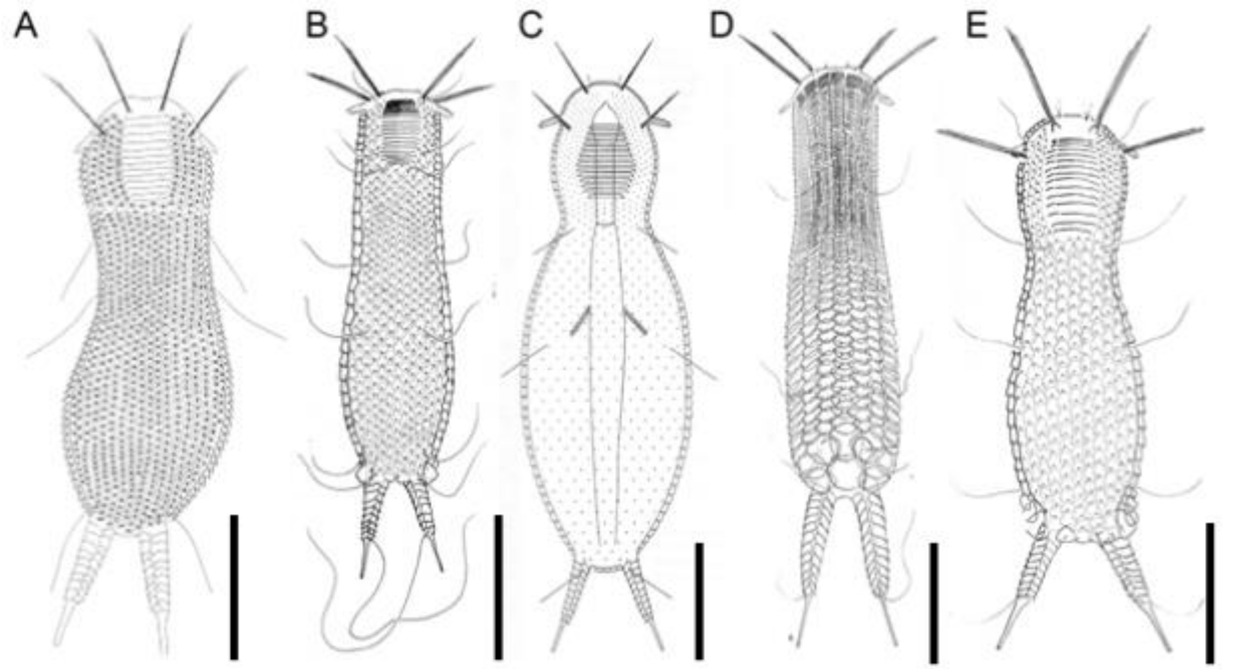

Figure 14 - Schematich draw of dorsal body. A. *X. texana* (modified from Todaro (1994). B. *X. transatlantica.* C - *X. simplex* (modified from Mock, 1979). D - *X. wikaea.* E - *X. affinis.* B, D-E - modified from Ruppert, 1979. Scale bars: 25 µm.

Table 12 - Geographic coordinates of *Xenotrichula affinis*

Country	Coordinates	Authors
Belgiun	(51°04'24.4"N , 3°40'50.5"E); (51°22'02.5"N, 3°21'36.3"E); (51°13'N, 02°53'E); (51°19'N, 03°21'E);	(Jouk et al., 1992; Hummon, 2010)
British Isles	(49°14'N; 02°12'W);	(Hummon, 2010)
Denmark	(55°04'N, 08°33'E); (55°05'21.7"N, 08°33'54.5"E);	(Mock, 1979; Hummon, 2010)
Eire	(52°15'N, 10°02'W);	Hummon 2010
England	(52°58'N, 00°29'E); (50°40'N, 01°53'W); (50°35'N, 03°20'W); (50°20'N, 04°26'W); (55°01'N, 01°26'W); (52°54'21.7"N , 4°39'00.3"W);	(Boaden, 1963; Hummon, 2010)
France	(44°39'47.0"N , 1°09'13.1"W); (44°37'44.0"N , 1°14'34.2"W); (44°38'00.3"N , 1°12'16.1"W); (44°34'03.7"N , 1°14'06.9"W); (43°26'01.5"N , 1°35'58.8"W); (43°39'48.5"N , 1°25'52.9"W); (43°48'56.4"N , 1°24'21.7"W); (44°53'40.8"N , 1°13'06.7"W); (45°34'20.5"N , 1°03'43.9"W); (45°00'06.2"N , 1°12'08.8"W); (44°47'41.9"N , 1°14'07.7"W); (45°04'N, 01°13'W); (45°41'47.8"N , 1°12'26.5"W); (44°42'N, 01°12'W); (44°36'N, 01°16'W); (44°38'N, 01°14'W); (43°25'N, 01°37'W); (43°39'N, 01°26'W); (45°41'N, 01°13'W); (45°04'N, 01°13'W); (44°42'N, 01°15'W); (44°39'N, 01°15'W); (44°45'N, 01°15'W); (44°39'N, 01°12'W); (44°39'N, 01°10'W); (45°33'N, 01°06'W); (44°44'N, 01°10'W); (44°42'N, 01°15'W); (44°38'N, 01°15'W); (45°01'N, 01°14'W); (43°49'N, 01°25'W); (43°50'N, 01°25'W); (43°39'N, 01°26'W); (51°00'N, 02°04'E); (44°33'N, 01°17'W); (44°42'N, 01°15'W); (44°50'N, 01°15'W); (48°33'N, 04°43'W); (44°38'N, 01°13'W); (43°57'N, 01°23'W); (43°24'N, 01°41'W); (44°39'N, 01°15'W); (48°22'57.4"N , 4°29'03.2"W); (44°40'00"N, 01°30'06"W);	(D'Hondt, 1966, 1968a, 1970; Mock, 1979; Hummon, 2010)
Germany	(54°25'00"N, 10°11'00"E); (55°02'N , 08°26'E); (54°48'N, 08°18'E); (54°25'N, 10°11'E); (55°02'11.6"N , 8°25'38.6"E); (54°47'54.8"N, 8°17'40.7"E);	(Remane, 1934; Mock, 1979; Hummon, 2010)
Israel	(32°04'N, 34°45'E);	(Hummon, 2010)
Japan	(26°12'N, 127°39'E);	(Hummon, 2010)
Scotland	(57°51'N, 05°28'W); (55°36'N, 04°42'W); (54°48'N, 04°56'W); (56°21'N, 02°48'W);	(Hummon, 2010)
USA	(38°47'N, 74°06'W); (38°47'N, 75°09'W); (41°32'N, 70°41'W); (41°40'N, 70°00'W); (41°31'N, 70°40'W); (42°42'N, 70°47'W); (42°41'N, 70°46'W); (43°00'N, 70°40'W); (43°32'N, 70°20'W); (40°50'N, 72°30'W); (43°00'N, 70°40'W); (42°40N, 70°50'W); (41°40'N, 70°00'W); (40°50'N, 72°30'W);	(Hummon, 1974a, 2010)
Wales	(52°53'N, 04°48'W);	(Hummon, 2010)

Xenotrichula arcassonensis (Ruppert, 1979) **nov. comb.**

Heteroxenotrichula arcassonensis Ruppert, 1979: 277, figs. 23-24.

Species with body up to 178 µm, subterminal mouth, pharynx length 37 µm. Head with a pair of small lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Dorsal body covered by nine rows of oval scales increasing in size anterior-posteriorly to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Furcal base covered by flat scales. Ventral body covered oval scales from U30 to the top of furca. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 15A).

Marine Realms: Temperate Northern Atlantic (Fig. 16).

Table 13 - Geographic coordinates of *Xenotrichula arcassonensis*

Country	Coordinates	Authors
France	(44°35'42"N, 01°35'09"W); (44°40'00"N, 01°30'06"W); (44°39'N, 01°10'W); (44°33'N, 01°17'W); (50°46'N, 01°35'E); (44°37'N, 01°17'W);	(Ruppert, 1979; Hummon, 2010)
Italy	(41 °15’N, 09°09’E); (39°14’N, 09°34’E); (40°58’N, 08°51’E); (41 °15’N, 09°09’E); (39°14’N, 09°34’E); (40°58’N, 08°51’E);	(Balsamo, Fregni & Tongiorgi, 1995; Hummon, 2010)

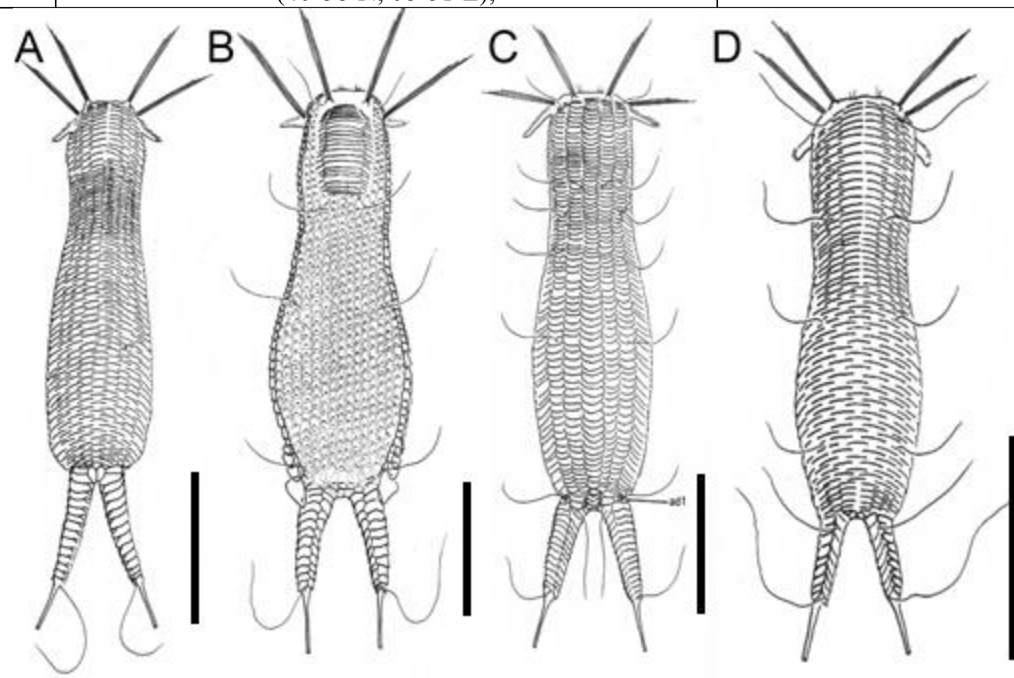

Figure 15 - Schematic draw of dorsal body. A. *X. arcassonensis.* B. *X. subterranea.* C - *X. squamosa.* D. *X. pygmea.* A-D, modified from Ruppert, 1979. Scale bars: 30 µm.

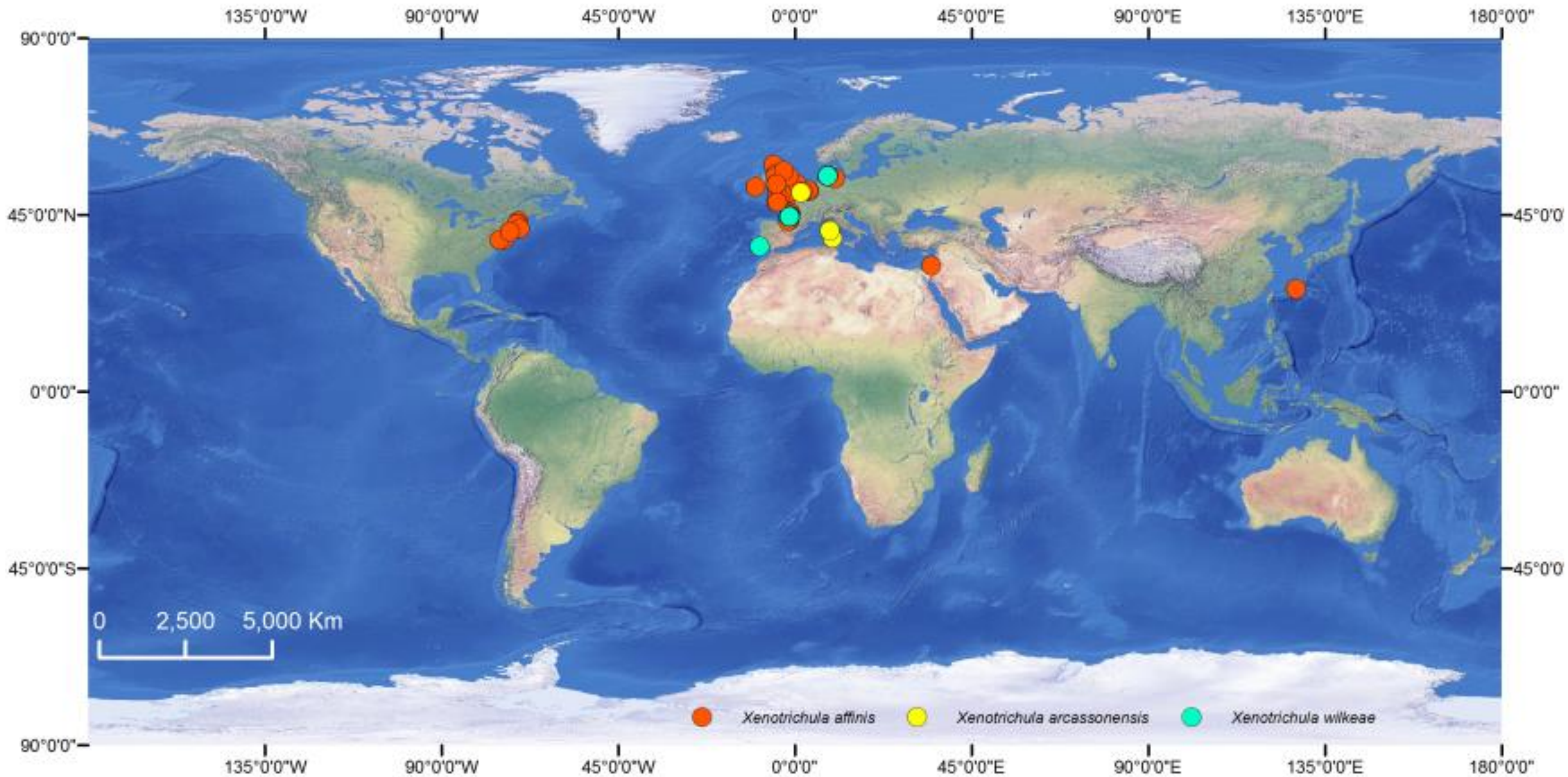

Figure 16 - Map of distribution of *X. affinis, X. arcassonensis* and *X. wilkeae.*

Xenotrichula subterranea Remane, 1934 **stat. rev.**

Syn. *Heteroxenotrichula subterranea* (Remane, 1934): 475, fig. 3

Syn. *Xenotrichula* sp. of Luporini, Magagnini & Tongiorgi 1971:449

Species with body up to 181 µm long, subterminal mouth, pharynx length 37.5 µm. Head with a pair of small lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Oval patch of 26 flat plates on the central area of the head. Dorsal body covered by two rows of pedunculated subterranea-type scales on each side of oval patch and 16 rows of subterranea-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly. Ventral body covered by flat plates from U51 to the top of furca. Three rows of plate at midventrally posterior part of the trunk. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 15B).

Marine Realms: Temperate Northern Atlantic, Temperate Northern Pacific, Western Indo-Pacific (Fig. 17).

Table 14 - Geographic coordinates of *Xenotrichula subterranea*

Country	Coordinates	Authors
British Isles	(49°14'N, 02°,12'W)	(Hummon, 2010)
Egypt	(31°,23'N, 27°,00'E)(31°,08'N, 33°,43'E)(31°,16'N, 27°,49'E)(31°,08'N, 33°,44'E)(31°,24'N, 27°,02'E)	(Hummon, 2010)
England	(51°,06'N, 04°,12'W)	(Hummon, 2010)
France	(44°35'42"N, 01°35'09"W)(50°,48'N, 01°,35'E)(44°,37'N, 01°,17'W)	(Ruppert, 1979; Hummon, 2010)
Germany	(54°,25'N, 10°,11'E)(54°25'00"N, 10°11'00"E)	(Remane, 1934; Hummon, 2010)
India	(17°32'55.2"N, 83°11'06.8"E)(11°29'24.0"N , 92°43'41.9"E)(17°42'09.0"N, 83°18'32.4"E)(17°32'55.2"N, 83°11'06.8"E)(17°,44'N , 83°,23'E)	(Rao & Ganapati, 1967, 1968; Naidu & Rao, 2004; Hummon, 2010)
Israel	(31°,48'N, 34°,38'E)(31°,44'N, 34°,36'E)(32°,24'N, 34°,52'E)	(Hummon, 2010)
Italy	(42°51'45.0"N , 10°25'13.9"E)(40°,22’N, 18°,18’E)(42°,51’N, 10°,25’E)(43°,44’N , 10°,16’E)(43°,32’N, 10°,17’E)(43°,38’N, 10°,15’E)(43°40'26.8"N , 10°16'16.2"E)(43°43'30.5"N , 10°16'43.4"E)(43°31'50.0"N , 10°18'09.6"E)	(Luporini, Magagnini & Tongiorgi, 1971; Balsamo, Todaro & Tongiorgi, 1992)
Portugal	(37°,01'N, 08°,57'W)	(Hummon, 2010)
Scotland	(57°,49'N, 05°,41'W)	(Hummon, 2010)
Somalia	(01°,57'N, 45°,11'E)	(Hummon, 2010)
USA	(33°,41'N, 118°,24'W)(43°,20'N, 124°,19'W)(43°,18'N, 124°,19'W)(42°,05'N, 124°,19'W)(45°,27'N, 123°,58'W)(46°,12'N, 124°,00'W)	(Hummon, 2010)
Wales	(52°,53'N, 04°,48'W)(53°,14'N, 04°,37'W)(53°,20'N,	(Boaden, 1963;

	04°,16'W)(52°54'21.7"N , 4°39'00.3"W)(53°13'38.0"N , 4°31'26.8"W)(53°20'24.4"N , 4°13'48.8"W)	Hummon, 2010)

Xenotrichula squamosa (Wilke, 1954)**nov. comb.**

Heteroxenotrichula squamosa Wilke, 1954: 522, figs. 17-18

Species with body up to 200 µm, subterminal mouth, pharynx length 51 µm. Head with a pair of small lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Dorsal body covered by nine rows of oval scales to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Furcal base covered by flat scales. Ventral body covered with seven rows of oval scales from U30 to the top of furca. Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 15C).

Remarks. According Leasi & Todaro (2008), *Heteroxenotrichula squamosa* presents circular muscles along the pharynx length, longitudinal muscles present along ventral, dorsal and lateral body, heliocoidal muscles are present from the head to half of length of intestine, and dorsoventral muscles are present along the intestinal tract, medial and lateral positions.

Marine Realms: Temperate Northern Atlantic, Temperate Southern Africa, Western Indo-Pacific, Tropical Atlantic, Temperate South America, Temperate Northern Pacific (Fig. 17).

Table 15 - Geographic coordinates of *Xenotrichula squamosa*

Country	Coordinates	Authors
Belgiun	(51°04'24.4"N , 3°40'50.5"E); (51°22'02.5"N, 3°21'36.3"E); (51°13'N, 02°53'E); (51°19'N, 03°21'E);	(Luporini, Magagnini & Tongiorgi, 1973; Hummon, 2010)
Brazil	(23°21'55.1"S, 44°56'30.2"W); (23°51'S, 45°17'W); (23°49'S, 45°27'W); (23°22'S, 44°57'W); (23°21'S, 44°56'W);	(Todaro & Rocha, 2005; Hummon, 2010)
British Isles	(49°28'N, 02°36'W);	(Hummon, 2010)
Cyprus	(34°50'N, 32°21'E); (34°58'N, 33°43'E);	(Hummon, 2010)
Denmark	(55°05'21.7"N, 8°33'54.5"E);	(Mock, 1979; Hummon, 2010)
Egypt	(31°23'N, 27°00'E); (31°07'N, 33°42'E); (31°08'N, 33°43'E); (30°48'N, 29°03'E); (31°16'N, 27°49'E); (30°59'N, 28°42'E); (27°10'N, 33°57'E); (28°05'N, 34°25'E); (31°03'N, 29°41'E); (30°53'N, 28°51'E); (31°20'N, 27°14'E); (31°14'N, 29°58'E);	(Hummon, 2010)
France	(43°16'48.8"N , 5°20'59.8"E); (44°39'N, 01°10'W); (44°36'N, 01°13'W); (41°42'N, 09°24'E); (42°08'N, 08°35'E); (42°50'N, 09°24'E); (43°11'N, 05°41'E); (42°29'N, 03°07'E); (42°43'N, 03°01'E); (42°42'N, 03°01'E);	(Swedmark, 1956; Hummon, 2010)
Germany	(55°02'11.6"N , 8°25'38.6"E); (54°58'16.5"N, 8°20'20.0"E); (55°01'N, 08°26'E); (55°02'N, 08°23'E); (54°10'N, 07°54'E);	(Mock, 1979; Hummon, 2010)
Greece	(36°11'N, 28°07'E); (37°56'N, 24°01'E); (36°16'N, 25°27'E); (35°11'N, 24°23'E); (39°53'N, 22°51'E); (35°19'N, 25°11'E); (35°21'N, 24°19'E); (35°01'N, 24°45'E); (35°06'N, 25°48'E); (35°05'N, 26°14'E); (35°20'N, 25°16'E); (37°48'N, 23°48'E); (36°17'N, 25°22'E); (40°15'N, 23°29'E); (40°02'N, 22°35'E); (35°02'N, 25°57'E); (34°59'N, 24°45'E); (35°11'N, 26°06'E); (36°12'N, 28°09'E); (35°14'N, 26°16'E);	(Leasi & Todaro, 2008; Hummon, 2010)
Israel	(31°53'N, 34°41'E); (29°30'N, 34°55'E); (31°48'N, 34°38'E); (32°19'N, 34°51'E);	(Hummon, 2010)
Italy	(40°50’N, 14°01’E); (43°40'26.1"N , 10°16'15.9"E); (42°48'03.9"N , 10°16'02.7"E); (42°51'45.0"N , 10°25'13.9"E); (43°03'12.7"N , 9°48'02.1"E); (40°47'N, 13°26'E); (41°13'N, 13°05'E); (40°47'N, 09°41'E); (42°39'N, 11°04'E); (39°41’N, 08°27’E); (41 °15’N, 09°09’E); (37°35’N, 12°53’E); (38°05’N, 13°03’E); (36°49’N, 14°28’E); (37°00’N, 15°17’E); (37°50’N, 15°17’E); (38°22’N, 14°59’E); (38°02’N, 14°04’E); (38°10’N, 12°45’E); (42°49’N, 10°44’E); (40°58’N, 17°18’E); (41°56’N, 16°00’E); (41°37’N, 15°54’E); (37°00’N, 15°17’E); (37°58’N, 16°07’E); (40°16’N, 17°51’E); (37°50’N, 15°17’E); (40°11’N, 16°44’E); (39°53’N, 18°07’E); (40°00’N, 17°59’E); (44°18’N, 08°29’E); (42°48’N, 10°15’E); (42°51’N, 10°25’E); (43°09’N, 10°40’E); (43°32’N, 10°17’E); (43°02’N, 09°47’E); (43°48’N, 07°46’E); (36°49’N, 14°28’E); (37°35’N, 12°53’E); (39°41’N, 08°27’E); (41 °15’N, 09°09’E); (42°39’N, 11°01’E); (42°24’N, 11°17’E); (41°31’N, 12°32’E); (40°50’N, 14°15’E); (42°45’N, 10°51’E); (41°59’N, 12°01’E); (40°43’N, 13°51’E); (40°44’N, 13°54’E); (42°24’N, 11°14’E); (40°50’N, 14°01’E); (40°45’N, 13°56’E); (40°45’N, 13°56’E); (38°27’N, 14°56’E); (38°22’N, 14°59’E); (41°57’N, 12°03’E); (40°54’N, 14°02’E); (42°20’N, 11°33’E); (42°56’N, 10°41’E); (40°25’N, 14°58’E); (40°47’N, 13°36’E); (40°00’N, 15°15’E); (40°49’N, 14°12’E); (41°15’N, 13°02’E); (38°02’N, 14°04’E); (41°30’N, 12°10’E); (41°13’N, 13°05’E); (42°02’N, 11°56’E); (38°10’N, 12°45’E); (41°14’N, 13°29’E); (41°12’N, 13°33’E); (41°17’N, 13°15’E); (40°47’N, 14°02’E); (38°05’N, 13°03’E); (40°44’N, 14°00’E); (42°27’N, 11°09’E); (41°47’N, 12°13’E); (40°52’N, 14°01’E); (40°00’N, 15°21’E); (41°03’N, 13°53’E); (42°49’N, 10°44’E); (42°39’N, 11°04’E); (43°44’N , 10°16’E); (43°44’N, 10°16’E); (40°25’N, 16°49’E); (40°12’N, 18°26’E); (41°54’N, 15°21’E); (40°22’N, 18°18’E);	(Wilke, 1954; Luporini, Magagnini & Tongiorgi, 1973; Balsamo, Todaro & Tongiorgi, 1992; Todaro, Fleeger & Hummon, 1995; Balsamo, Fregni & Tongiorgi, 1996; Todaro et al., 2003; Hummon, 2010; Kånneby, Todaro & Jondelius, 2012)
Portugal	(37°06'N, 08°33'W);	(Hummon, 2010)
Scotland	(55°41'43.9"N , 4°53'57.7"W); (57°24'N, 01°51'W); (57°49'N, 05°41'W); (57°51'N, 05°28'W); (55°37'N, 04°44'W); (55°36'N, 04°42'W); (56°23'N, 02°48'W); (58°30'N, 04°22'W); (56°21'N, 02°48'W);	(Kisielewski, 1975; Hummon & Todaro, 2010)
South Africa	(28°23'S, 32°25'E);	(Todaro et al., 2011)
Spain	(38°41'N, 01°28'E);	(Hummon, 2010)
USA	(42°53'43" N, 70°49'13" W); (43°00'N, 70°40'W); (41°40'N, 70°00'W); (41°30'N, 70°40'W); (41°20'N, 70°30'W); (41°31' N, 70°40'W); (41°32' N, 70°41 W); (41°32' N, 70°41' W); (41°33' N, 70°40' W); (27°43'N, 97°09'W); (26°06'N, 97°09'W); (34°38'30"N, 77°05'23"W); (26°13'N, 80°05'W); (27°48'N, 80°25'W); (27°32'N, 82°43'W); (26°43'N, 82°15'W); (41°32'N, 70°41'W); (41°45'N, 70°26'W); (43°00'N, 70°40'W); (41°21'N, 70° 29'W); (41°32'N, 70°41'W); (41°40'N, 70°00'W); (41°31'N, 70°40'W); (41°33'N, 70°40'W); (42°50'N, 70°48'W); (34°38'N, 77°05'W); (33°20'N, 79°10'W); (27°43'N, 97°09'W); (27°05'N, 97°23'W); (33°45'N, 118°08'W); (38°36'N, 75°04'W); (30°17'N, 87°08'W); (29°40'N, 85°23'W); (43°29'N, 70°22'W); (42°53'N, 70°49'W);	(Hummon, 1969, 1974a, 2010; McGeary, 1974; Ruppert, 1979; Todaro, Fleeger & Hummon, 1995)
Wales	(51°31'N, 04°03'W);	(Hummon, 2010)

Xenotrichula pygmaea Remane, 1934 **stat. rev.**

Syn. *Heteroxenotrichula pygmaea* (Remane, 1934): 477, fig. 5

Syn. *Xenotrichula flandrensis* d'Hondt, 1968a: 221

Species with body up to 110 µm, subterminal mouth, pharynx length 26.3 µm. Head with a pair of small lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Dorsal body covered by nine rows of oval scales to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Furcal base covered by flat scales. Probably scales plates on the ventral second half body (Ruppert 1979). Locomotory cirri covering the anterior part of ventral side, different size of cirri and divided (U11) in two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present.

Remarks: We rejected the synonymizing of *X. pygmaea* with *Xenotrichula* sp. A of Schmidt, 1974:61. Schimidt (1974) does not provide any morphological information to support the synonymy proposed by Ruppert (1979). By this reason we suggest this species continues as undetermined morphotype (Fig. 15D).

Marine Realms: Temperate Northern Atlantic, Tropical Eastern Pacific, Western Indo-Pacific, Tropical Atlantic, Temperate South America, Temperate Northern Pacific, Eastern Indo-Pacific, Central Indo-Pacific (Fig. 17).

Table 16 - Geographic coordinates of *Xenotrichula pygmaea*

Country	Coordinates	Authors
Bahamas	(22°,37'N, 73°,30'W)	(Hummon, 2010)
Brazil	(23°12'23"S, 44°39'30.4"W)(23°,51'S, 45°,25'W)	(Todaro & Rocha, 2005; Hummon, 2010)
Bulgary	(43°,12'N, 27°,57'E)	(Valkanov, 1957; Hummon, 2010)
Egypt	(31°,16'N, 27°,49'E)(31°,06'N, 29°,43'E)(31°,06'N, 29°,43'E)(30°,19'N, 32°,18'E)(27°,07'N, 33°,49'E)(26°,03'N, 33°,54'E)(29°,08'N, 32°,39'E)(27°,10'N, 33°,57'E)(27°,44'N, 34°,12'E)(29°,14'N, 32°,53'E)(28°,05'N, 34°,25'E)(27°,56'N, 34°,23'E)(27°,50'N, 34°,14'E)(29°,36'N, 32°,40'E)(28°,50'N, 34°,36'E)(27°,43'N, 34°,15'E)(29°,52'N, 32°,38'E)	(Hummon, 2010)
France	(44°,43'N, 01°,06'W)(48°,44'N, 04°,05'W)(50°,46'N, 01°,35'E)(51°,05'N, 02°,29'E)(43°,31'N, 03°,57'E)(51°04'19.4"N, 2°29'14.3"E)	(D'Hondt, 1968b; Hummon, 2010)
Galapagos	(00°,45'S, 90°,17'W)	(Hummon, 2010)
Germany	(55°02'11.6"N , 8°25'38.6"E)(54°58'16.9"N, 8°20'20.1"E)(55°03'02.1"N , 8°26'28.4"E)(55°,02'N , 08°,26'E)(55°,03'N, 08°,26'E)(55°,01'N, 08°,26'E)(54°,25'N, 10°,11'E)(55°,02'N, 08°,23'E)(54°25'00"N, 10°11'00"E)	(Remane, 1934; Mock, 1979; Hummon, 2010)
Greece	(35°,11'N, 24°,23'E)(36°,16'N, 25°,27'E)(35°,19'N, 25°,11'E)(35°,01'N, 24°,45'E)(36°,21'N, 27°,57'E)	(Hummon, 2010)
Hawaii	(21°,38'N, 157°,55'W)	(Hummon, 2010)
Israel	(31°,40'N, 34°,34'E)(31°,44'N, 34°,36'E)(31°,53'N, 34°,41'E)(29°,32'N, 34°,57'E)	(Hummon, 2010)
Italy	(40°,12'N, 18°,26'E)(45°,43'N, 13°,22'E)(45°,45'N, 13°,31'E)(40°,22'N, 18°,18'E)(39°,48'N, 18°,22'E)(41°,03'N, 13°,53'E)(40°,44'N, 13°,54'E)(40°,52'N, 14°,01'E)(40°,26'N, 09°,50'E)(40°,32'N, 14°,04'E)(40°,25'N, 14°,58'E)(40°,44'N, 14°,00'E)	(Hummon, 2010)
Japan	(24°,10'N, 123°,53'E)(24°,09'N, 124°,03'E)	(Hummon, 2010)
Portugal	(38°,30'N, 28°,17'W)(37°,44'N, 25°,36'W)	(Hummon, 2010)
USA	(41°31'N, 70°40'W)(41°32'N, 70°41'W)(41°32'N, 70°41'W)(41°33'N, 70°40'W)(41°35'N, 70°39'W)(34°38'30"N, 77°05'23"W)(27°28'N, 97°16'W)(38°,47'N, 75°,09'W)(26°,13'N, 80°,05'W)(27°,48'N, 80°,25'W)(24°,39'N, 81°,16'W)(25°,43'N, 80°,09'W)(25°,26'N, 80°,12'W)(24°,41'N, 81°,05'W)(41°,32'N, 70°,41'W)(41°,45'N, 70°,26'W)(41°,21'N, 70°, 29'W)(41°,32'N, 70°,41'W)(41°,31'N, 70°,40'W)(42°,42'N, 70°,47'W)(42°,41'N, 70°,46'W)(41°,33'N, 70°,40'W)(41°,35'N, 70°,39'W)(34°,38'N, 77°,05'W)(40°,50'N, 72°,30'W)(43°,20'N, 124°,19'W)(43°,21'N, 124°,20'W)(44°,00'N, 124°,08'W)(45°,29'N, 123°,55'W)(45°,26'N, 123°,57'W)(44°,55'N, 124°,01'W)(44°,10'N, 124°,07'W)(29°,55'N, 81°,17'W)(27°,28'N, 97°,16'W)(48°,27N, 122°,59'W)	(Hummon, 1969, 2010; Mock, 1979; Todaro, Fleeger & Hummon, 1995)
Virgin Islands	(18°,21'N, 64°,52'W)(18°,20'N, 64°,51'W)	(Hummon, 2010)
Wales	(51°,32'N, 04°,04'W)	(Hummon, 2010)

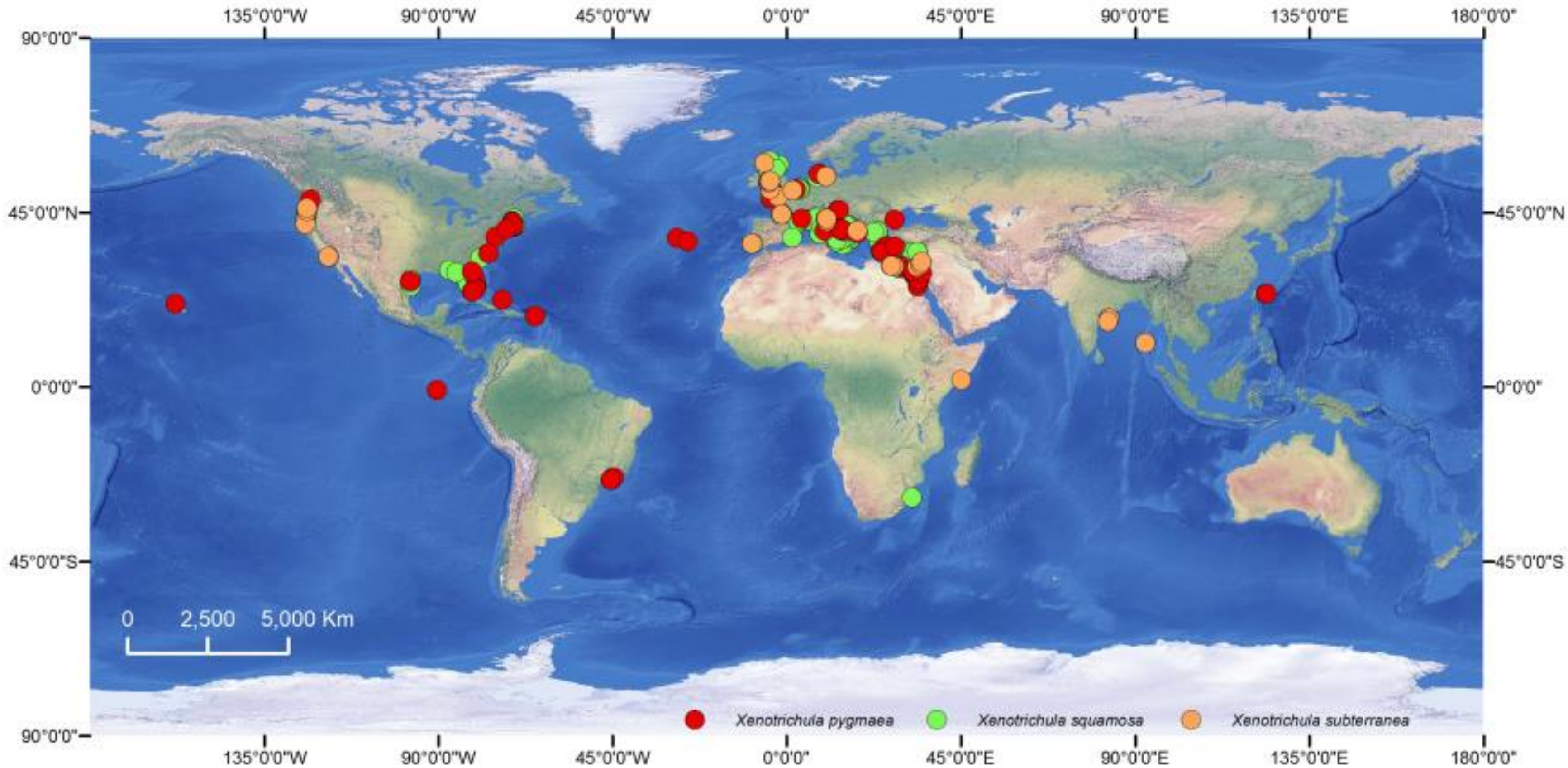

Figure 17 - Map of distribution of *X. pygmaea*, *X. squamosa* and *X. subterranea*.

Genus *Minutuxenotrichula*, gen. nov.

Type-species *Minutuxenotrichula tentaculata* (Rao & Ganapati, 1968)

Diagnosis: Xenotrichulinae with head presenting long tentacles which can possess scales and/or cilia. Cephalion region covered by scales. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Short pharynx. Dorsal body covered by minute elliptical scales. Hydrofoil scales absent. Ventral side of body without scales. Furca bases covered by flat scales. Ventral locomotory cirri of different sizes anteriorly, the first portion of head can be all covered by cirri and divided in two longitudinal bands to the PHJIn, a pair of locomotory cirri on the middle trunk .

Etymology: From Latin “minutus”, referring to the small scales covering the dorsal body.

Minutuxenotrichula tentaculata (Rao & Ganapati, 1968) **nov. comb.**

Syn. *Xenotrichula tentaculata* Rao & Ganapati, 1968: 51, figs. 27-28

Species with body up to 220 µm, anterior mouth, pharynx length 50 µm. Head with a pair of long lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle. Dorsal body covered by 11 rows of minute elliptical scales to the top of furca. Furcal base covered by flat scales. Ventral body without scales. Locomotory cirri two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 18A).

Marine Realms: Western Indo-Pacific (Fig. 19).

Table 17 - Geographic coordinates of *Minutuxenotrichula tentaculata*

Country	Coordinates	Authors
Indonesia	(8°,46'S, 115°,10'E)	Hummon 2010
India	(17°32'55.2"N, 83°11'06.8"E)(19°,52'N, 86°,07'E)(19°,51'N, 86°,03'E)(19°,46'N, 85°,46'E)(19°,47'N, 85°,49'E)(19°,52'N, 86°,07'E)(17°32'55.2"N, 83°11'06.8"E)(11°29'24.0"N , 92°43'41.9"E)(19°33'12.2"N , 85°18'26.0"E)(19°,15'N, 84°,55'E)(19°,52'N, 86°,07'E)(19°,51'N, 86°,03'E)(17°,44'N, 83°,20'E)(17°,41'N, 83°,17'E)(17°,43'N, 83°,20'E)(19°,46'N, 85°,46'E)(19°,47'N, 85°,49'E)(19°,48'N, 85°,51'E)(17°,42'N, 83°,19'E)	(Rao & Ganapati, 1968; Rao, 1969; Naidu & Rao, 2004; Hummon, 2010)

Minutuxenotrichula laccadivensis (Rao, 1991): **nov. comb.**

Xenotrichula laccadivensis Rao, 1991: 76, fig.5

Species with body up to 225 µm, anterior mouth, pharynx length 65 µm. Head with a pair of long lateral tentacles. Neck weakly marked. Transversal row of cilia on each side of mouth extending to total ventral length of tentacle (?). Dorsal body covered by six rows on the neck and 11 of minute elliptical scales to the top of furca. Furcal base covered by flat scales. Ventral body without scales. Locomotory cirri two longitudinal bands to the PHJIn region. One pair of locomotory cirri tufts at middle trunk and one pair on the posterior gut. Paired testes are present (Fig. 18B-C).

Marine Realms: Western Indo-Pacific (Fig. 19)

Table 18 - Geographic coordinates of *Minutuxenotrichula laccadivensis*

Country	Coordinates	Authors
India	(10°33'N, 72°38'E)	(Rao, 1991; Naidu & Rao, 2004; Hummon, 2010)

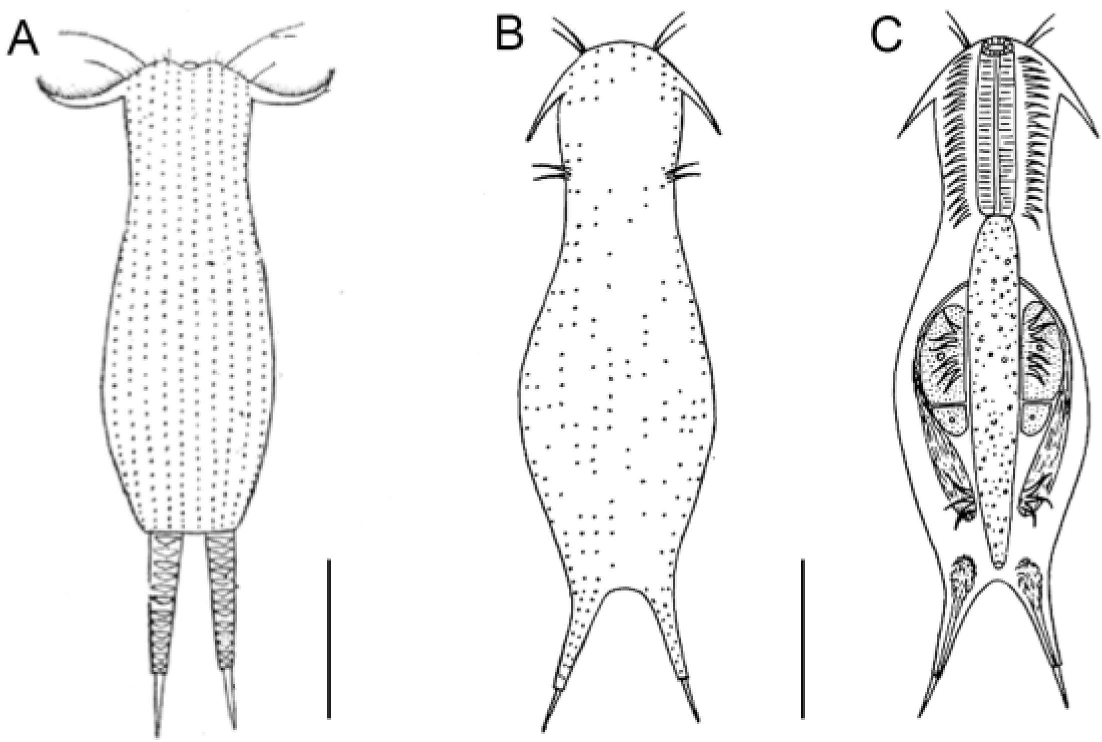

Figure 18 - Schematic draw. A- *M. tentaculata* (modified from Rao and Ganapati, 1968). B - C, *M. laccadivensis* (modified from Rao, 1991). B - Dorsal body. C - Ventral and internal body. Scales bars: 50 µm.

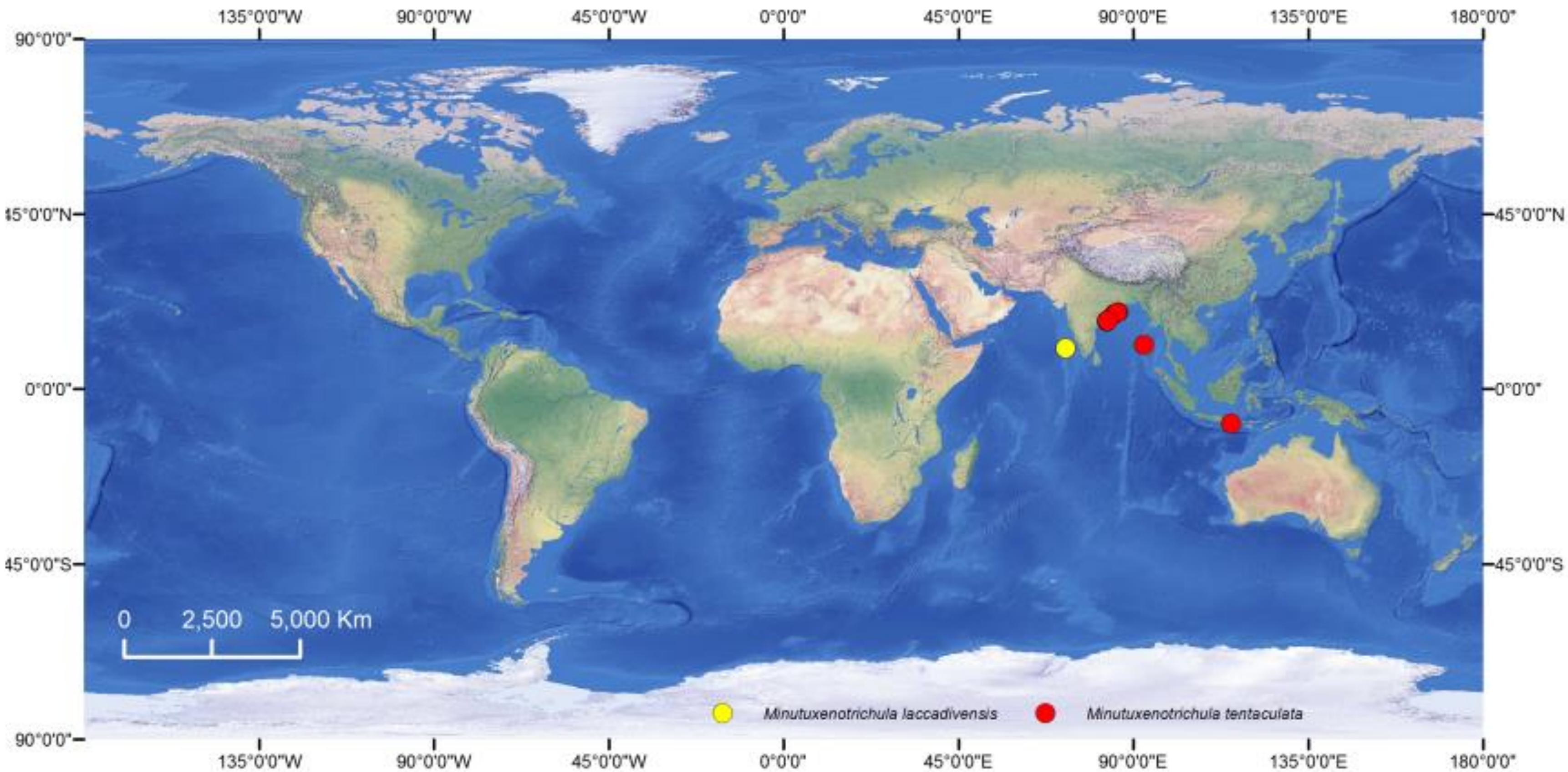

Figure 19 - Map of distribution of *Minutuxenotrichula* species.

Subfamily Rotunduxenotrichulinae **subfam. nov.**

Diagnosis: Xenotrichulidae with somewhat short body, semicircular in cross section, head weakly marked and furca what is distinctly delimited from the trunk. Rounded head. Two or three pairs of sensorial cirri on the head. Cephalion region covered by scales or the region can be naked. An oval patch of transversal flat plates can be present on the dorsal head. Short pharynx. Dorsal body covered by intermedia-type or hexagonal scales, with one pedunculum or two. Furca bases covered by flat scales. Hydrofoil scales usually present on the ventrolateral body. Ventrally the body can be covered by scales and can present longitudinal plates on the middle trunk to the end. Triangular area of flat scales can be present on the end ventral body. Ventral locomotory cirri with equal sizes anteriorly, two longitudinal bands extending from the head to the PHJIn, a pair of locomotory cirri on the middle trunk. Testis with caudal anastomosis. X-organ present.

Etymology: From the Latin “rotundun”, referring to the rounded head of species of this subfamily.

Type-Genus *Rotunduxenotrichula*, gen. nov.

Diagnosis. Same diagnosis of subfamily.

Type-species *Rotunduxenotrichula intermedia* (Remane, 1934): 477, fig. 6 **nov. comb.**

Syn. *Xenotrichula intermedia*, Remane 1934: 477

Syn. *Xenotrichula beauchampi* Lévi, 1950:39

Syn. *Xenotrichula beauchampi* var. *angusta* d'Hondt 1966a: 6

Syn. *Xenotrichula beauchampi* var. *maritima* d'Hondt 1970: 10

Syn. *Xenotrichula punctata* of Swedmark 1956:86

Species with body up to 287 µm, terminal mouth, pharynx length up to 67.5 µm. Rounded head. Neck well marked. Two hard projections on each side of mouth (old Bidentate hypostomium). Three pairs of sensorial cirri on the head. Dorsal body covered by 16-20 rows of intermedia-type scales to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Furcal base covered by flat scales. Ventral body covered by nine rows of intermedia-type scales to the top of furca. Triangular area of flat scales present on the end ventral body. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present. Bilobed X-organ present (Fig. 20).

Marine Realms: Temperate Northern Atlantic, Temperate Northern Pacific, Western Indo-Pacific, Tropical Eastern Pacific, Tropical Atlantic, Temperate South America (Fig, 21).

Remarks: We rejected the synonymizing of *R. intermedia* with *Xenotrichula carolinensis* ssp. *syltensis* Mock, 1979 as suggested by Hummon and Todaro, 2010. Mock (1979) provided good morphological information such as the lateral of ventral body covered by scales and a crossing of intermedia-type scales on the ventral body at point of the pair of locomotory cirri tufts, a characteristic of *R. carolinenses* (Ruppert, 1979). Due to the cosmopolitan distribution of *R. intermedia*, the cryptic potential of this species had been investigated; Todaro (1996) founded high genetic diversity between three populations of *R. intermedia;* Leasi and Todaro (2008) also found two different muscular system conditions between these populations. Chapter 2 of this thesis presents a complete description of a new muscular system and the comparison between the observed data and the results provided by Leasi and Todaro (2008, 2009).

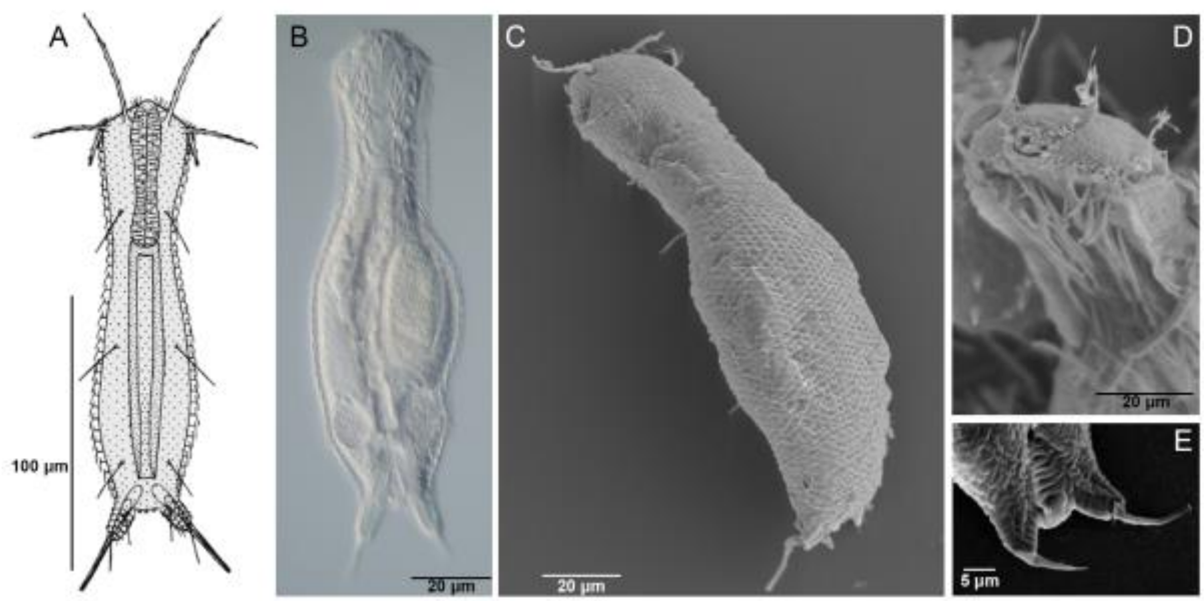

Figure 20 - *R. intermedia.* A - Schematic draw of dorsal body (modified from Hummon, 2008). B - DIC microphotography of ventral body. C-E SEM microphotography. C - Dorsal body. D - Close-up on the mouth. E. Close-up on the triangular area of flat scales on the ventral end body.

Table 19 - Geographic coordinates of *Rotunduxenotrichulaintermedia*

Country	Coordinates	Authors
Algeria	(35°,42'N, 00°,52'W)(35°,39'N, 01°,03'W)(35°,43'N, 00°,51'W)(35°,34'N, 01°,09'W)(36°,36'N, 02°,26'E)(35°,43'N, 00°,45'W)	(Hummon, 2010)
Belgium	(51°22'02.5"N, 3°21'36.3"E)(51°08'02.9"N , 2°40'10.5"E)(51°15'02.6"N , 2°57'41.3"E)(51°,14'N, 02°,56'E)(51°,06'N, 02°,34'E)(51°,19'N, 03°,21'E)	(Jouk et al., 1992; Hummon, 2010)
Brazil	(23°48'17.08"S, 45°23'48.90"W)(22°31'41.50"S, 41°56'44.96"W)(22°23'50.83"S, 41°47'9.46"W)(17°54'24.58"S, 39°22'3.51"W)(23°21'55.1"S, 44°56'30.2"W)(23°22'39.1"S, 44°57'18.2'W)(23°22'57.5"S, 45°03'41.1"W)(23°,51'S, 45°,25'W)(23°21'55.1"S, 44°56'30.2"W)(23°,46'S, 45°,42'W)(23°,46'S , 45°,41'W)(23°,48'S, 45°,32'W)(23°,22'S, 45°,03'W)	(Todaro & Rocha, 2005; Hummon, 2010); Present Study
British Isles	(49°14'N, 02°,12'W)(49°,11'N, 02°,02'W)(49°,10'N, 02°,11'W)	(Hummon, 2010)
Bulgary	(43°,12'N, 27°,57'E)(42°,26'N, 27°,42'E)(43°,12'N, 27°,57'E)(42°,26'N, 27°,42'E)	(Valkanov, 1957; Hummon, 2010)
Denmark	(55°05'21.7"N, 8°33'54.5"E)(55°,04'N, 08°,33'E)	(Mock, 1979; Hummon, 2010)
Ecuador	(01°,33'S, 80°,48'W)	(Hummon, 2010)
Egypt	(30°,50'N, 29°,15'E)(31°,12'N, 29°,56'E)(30°,54'N, 29°,29'E)(30°,59'N, 28°,42'E)(31°,07'N, 33°,42'E)(30°,48'N, 29°,03'E)(31°,14'N, 29°,58'E)(31°,06'N, 29°,43'E)	(Hummon, 2010)
Eire	(53°57'N, 10°,11'W)(51°,50'N, 10°,13'W)	(Hummon, 2010)
England	(49°,56'N, 06°,18'W)(49°,58'N, 06°,17'W)(49°,57'N, 06°,16'W)(49°,56'N, 06°,19'W)(55°,01'N, 01°,26'W)(52°,58'N, 00°,29'E)(50°,35'N, 03°,20'W)(49°,56'N, 06°,18'W)(51°,11'N, 03°,23'W)(52°,56'N, 00°,19'E)(49°,58'N, 06°,17'W)(52°,27'N, 01°,31'E)(49°,57'N, 06°,16'W)(49°,56'N, 06°,19'W)(49°,54'N, 06°,18'W)(54°,39'N, 01°,11'W)(51°,56'N, 01°,06'E)(54°,26'N, 00°,32'W)(50°,35'N, 01°,56'W)(51°,30'N, 00°,40'W)(51°,50'N, 01°,07'E)(50°,18'N, 04°,14'W)	(Hummon & Warwick, 1990; Hummon, 2010)
France	(44°40'58.9"N , 1°06'51.3"W)(44°39'47.7"N , 1°09'15.3"W)(44°45'43.9"N , 1°10'39.8"W)(50°,48'N, 01°,35'E)(46°,07'N, 01°,10'W)(44°,42'N, 01°,12'W)(50°,26'N, 01°,33'E)(48°,44'N, 04°,00'W)(44°,44'N, 01°,07'W)(43°,25'N, 01°,37'W)(48°,40'N, 03°,56'W)(50°,09'N, 01°,29'E)(44°,43'N, 01°,06'W)(44°,42'N, 01°,15'W)(44°,44'N, 01°,13'W)(49°,18'N, 00°,28'W)(49°,15'N, 00°,12'W)(44°,39'N, 01°,05'W)(50°,38'N, 01°,34'E)(44°,42'N, 01°,15'W)(44°,38'N, 01°,07'W)(50°,04'N, 01°,23'E)(50°,20'N, 01°,32'E)(48°,42'N, 04°,00'W)(44°,45'N, 01°,09'W)(44°,39'N, 01°,10'W)(44°,37'N, 01°,17'W)(45°,41'N, 01°,13'W)(44°,39'N, 01°,15'W)(44°,38'N, 01°,14'W)(44°,38'N, 01°,15'W)(44°,33'N, 01°,17'W)(44°,42'N, 01°,15'W)(44°,38'N, 01°,13'W)(44°,39'N, 01°,15'W)(50°,46'N, 01°,35'E)(51°,05'N, 02°,29'E)(42°,42'N, 03°,01'E)(48°43'37.5"N, 3°59'15.6"W)(42°,38'N, 03°,01'E)(42°,42'N, 03°,01'E)(42°,38'N, 03°,01'E)(49°,18'N, 00°,28'W)(49°,15'N, 00°,12'W)(44°39'47.0"N , 1°09'13.1"W)(43°26'01.5"N , 1°35'58.8"W)(45°41'47.8"N , 1°12'26.5"W)(46°06'03.5"N , 1°06'42.6"W)(48°44'25.6"N , 4°00'02.7"W)(48°43'24.9"N , 3°59'31.9"W)(48°41'17.1"N , 3°55'33.2"W)(48°38'52.0"N , 4°18'29.7"W)(48°35'49.9"N, 4°34'10.7"W)(48°43'06.6"N , 4°00'47.8"W)(48°43'35.5"N , 4°00'08.0"W)(44°39'47.0"N , 1°09'13.1"W)(48°41'17.1"N , 3°55'33.2"W)(43°16'48.8"N , 05°20'59.8"E)	(Levi, 1950; Delamare-Deboutteville, 1954; Swedmark, 1956; Kaplan, 1958; D'Hondt, 1966, 1970; Kisielewski, 1988; Hummon, 2010)
Germany	(54°25'00"N, 10°11'00"E)(55°02'11.6"N , 8°25'38.6"E)(54°54'47.8"N , 8°21'51.4"E)(55°00'17.9"N , 8°23'49.0"E)(54°47'57.7"N, 8°17'41.5"E)(55°,00'N, 08°,23'E)(55°,01'N, 08°,26'E)(54°,10'N, 07°,54'E)(55°,03'N, 08°,26'E)(55°,01'N, 08°,26'E)(54°,54'N, 08°,22'E)(54°,48'N, 08°,18'E)(54°,25'N, 10°,11'E)(55°,02'N , 08°,26'E)	(Remane, 1934; Mock, 1979; Hummon, 2010)
Greece	(40°,07'N, 22°,33'E)(40°,18'N, 23°,41'E)(39°,56'N, 22°,42'E)(40°,12'N, 23°,47'E)(35°,20'N, 25°,05'E)(36°,12'N, 28°,09'E)(35°,11'N, 24°,23'E)(37°,56'N, 24°,01'E)(40°,17'N, 23°,40'E)(40°,05'N, 23°,18'E)(39°,53'N, 22°,51'E)(40°,21'N, 23°,53'E)(35°,19'N, 25°,11'E)	(Hummon, 2010)
India	(17°32'55.2"N, 83°11'06.8"E)(11°29'24.0"N , 92°43'41.9"E)(17°32'55.2"N, 83°11'06.8"E)(17°42'09.0"N, 83°18'32.4"E)	(Rao & Ganapati, 1968; Naidu & Rao, 2004) 2004)

Isle Of Man	(54°,05'N,04°,46'W)	(Hummon, 2010)
Israel	(32°,24'N, 34°,52'E)(31°,44'N, 34°,36'E)(32°,43'N, 34°,56'E)(33°,02'N, 35°,05'E)	(Hummon, 2010)
Italy	(42°51'45.0"N , 10°25'13.9"E)(42°44'55.0"N , 10°14'18.4"E)(42°21'04.0"N , 10°55'13.7"E)(39°,08’N, 09°,33’E)(40°,26’N, 09°,50’E)(40°,35’N, 08°,11’E)(40°,36’N, 08°,17’E)(37°,16’N, 13°,35’E)(37°,06’N, 14°,06’E)(37°,45’N, 12°,28’E)(36°,49’N, 14°,28’E)(37°,35’N, 12°,53’E)(41°11'N, 14°51'E)(36°51'N, 76°43'W)(30°17'N, 87°08'W)(40°47'N, 09°41'E)(40°09'N, 17°57'E)(44°28'N, 12°28'E)(42°02'16.47"N, 14°51'02.08"E)(42°01'N, 14°52'E)(45°,35’N, 12°,52’E)(42°,21’N, 14°,17’E)(42°,25’N, 14°,09’E)(41°,11’N, 14°,51’E)(44°,28’N, 12°,18’E)(42°,47’N, 13°,53’E)(43°,42’N, 13°,13’E)(41°,37’N, 15°,54’E)(41°,52’N, 16°,10’E)(42°,06’N, 15°,29’E)(40°,09’N, 17°,57’E)(43°,54’N, 08°,05’E)(44°,05’N, 09°,55’E)(44°,13’N, 09°,13’E)(44°,16’N, 09°,23’E)(37°,16’N, 13°,35’E)(40°,35’N, 08°,11’E)(40°,36’N, 08°,17’E)(37°,16’N, 13°,35’E)(37°,06’N, 14°,06’E)(37°,45’N, 12°,28’E)(42°,44’N, 10°,17’E)(42°,21’N, 10°,54’E)(42°,19’N, 10°,55’E)(41°,51’N, 12°,12’E)(42°,27’N, 11°,09’E)(41°,44’N, 12°,16’E)(39°,52’N, 15°,46’E)(39°,08’N, 09°,33’E)(40°,49’N, 14°,12’E)(42°,56’N, 10°,41’E)(45°,37’N, 13°,02’E)(45°,32’N , 12°,45’E)(45°,40’N, 13°,07’E)(41°,51’N, 16°,11’E)(43°,58’N, 12°,55’E)(41°,56’N, 16°,00’E)(39°,50’N, 18°,13’E)(43°,09’N, 10°,40’E)(43°,44’N , 10°,16’E)(42°,51’N, 10°,25’E)(43°,32’N, 10°,17’E)(43°,38’N, 10°,15’E)(36°,49’N, 14°,28’E)(37°,35’N, 12°,53’E)(42°,49’N, 10°,44’E)(41°,31’N, 12°,32’E)(42°,45’N, 10°,51’E)(41°,47’N, 12°,13’E)(41°,57’N, 12°,03’E)(40°,26’N, 09°,50’E)(41°,30’N, 12°,10’E)(42°,02’N, 11°,56’E)(41°,12’N, 13°,33’E)(43°,38’N, 10°,15’E)(43°40'26.1"N , 10°16'15.9"E)(43°42'07.1"N , 10°16'50.7"E)(45°46'37.6"N , 13°34'24.2"E)(39°,52’N, 15°,46’E	(Gerlach, 1953; Wilke, 1954; Schrom, 1966; Luporini, Magagnini & Tongiorgi, 1973; Balsamo, Todaro & Tongiorgi, 1992; Todaro, Fleeger & Hummon, 1995; Todaro et al., 1996, 2003, Leasi & Todaro, 2008, 2009; Hummon, 2010)
Kuwait	(29°23'38.58"N, 48°24'07.37"E)	(Leasi & Todaro, 2009; Kånneby, Todaro & Jondelius, 2012, 2013)
Northern Ireland	(54°,33'N, 05°,33'W)(55°,03'N, 06°,02'W)(54°,33'N, 05°,33'W)	(Boaden, 1966; Hummon, 2010)
Norway	(69°,35'N, 18°,02'E)(69°,37'N, 18°,48'E)	(Hummon, 2010)
Poland	(53°56'48.7"N , 14°28'20.6"E)(54°,55'N, 18°,21'E)(54°,43'N, 17°,12'E)(54°44'57.7"N , 18°40'09.7"E)(54°,22'N, 19°,28'E)(54°,15'N, 15°,17'E)(53°,57'N, 14°,28'E)(37°,01'N, 08°,57'W)(53°55' N, 14°15' E)(53°55' N, 14°26' E)(53°55' N, 14°26' E)(54°08' N, 15°17' E)(54°09' N, 15°24' E)(54°43' N, 17°13' E)(54°47'72" N, 17°43'79" E)(54°48'84" N, 17°50'63" E)(54°48' N, 18°22' E)(54°44' N, 18°34' E)(54°29'58" N, 18°34'78" E)(54°77'10" N, 18°34'06" E)(54°22' N, 19°26' E)(54°,55'N, 18°,21'E)(54°,43'N, 17°,12'E)(54°44'57.7"N , 18°40'09.7"E)	(Kisielewski, 1975; Hummon, 2008, 2010; KOLICKA et al., 2014)
Romania	(44°,03'N, 28°,38'E)(44°,10'N, 28°,39'E)(44°,15'N, 28°,37'E)(44°,03'N, 28°,38'E)	(Rudescu, 1966; Hummon, 2010)
Scotland	(57°,20'N, 01°,56'W)(57°,40'N, 01°,59'W)(56°,28'N, 02°,49'W)(54°,43'N, 04°,57'W)(55°,56'N, 03°,06'W)(57°,34'N, 01°,50'W)(54°,48'N, 04°,56'W)(57°,24'N, 01°,51'W)(57°,49'N, 05°,41'W)(57°,51'N, 05°,28'W)(55°,36'N, 04°,42'W)(55°41'43.9"N , 4°53'57.7"W)	(Hummon, 1975, 2010)
Tunisia	(35º30'57" N, 11º03'00" E)	(Kånneby, Todaro & Jondelius, 2012, 2013)
USA	(42°53'59.13"N, 70°48'38.12"W)(42°55'58.84"N, 70°47'48.65"W)(42°52'39.88"N, 70°48'56.21"W)(42°53'59.13"N, 70°48'38.12"W)(42°54'26.14"N, 70°48'34.74"W)(42°55'58.84"N, 70°47'48.65"W)(42°57'0.35"N, 70°47'8.79"W)(43°10'30.90"N, 70°36'30.90"W)(27°27'40.39"N, 80°18'46.13"W)(27°39'13.16"N, 80°21'17.83"W)(29°48'N, 84°40'W)(29°41'N, 84°44'W)(29°37'N, 84°52'W)(29°40'N, 85°23'W)(30°06'N, 85°46'W)(30°17'N, 87°08'W)(30°13'N, 87°40'W)(30°14'N, 88°09'W)(30°32'N, 88°55'W)(29°11'N, 94°57'W)(27°50'N, 97°03'W)(27°43'N, 97°09'W)(26°06'N, 97°09'W)(30°,14'N, 88°,09'W)(30°,13'N, 87°,40'W)(33°,45'N, 118°,08'W)(38°,45'N, 75°,04'W)(30°,19'N, 86°,09'W)(30°,06'N, 85°,46'W)(29°,41'N, 84°,44'W)(42°,37'N, 70°,37'W)(42°,34'N, 70°,45'W)(38°,37'N, 76°,30'W)(38°,21'N, 76°,23'W)(38°,42'N, 76°,31'W)(30°,32'N, 88°,55'W)(39°,38'N, 74°,11'W)(29°,11'N, 94°,57'W)(36°,54'N, 76°,07'W)(37°,02'N, 76°,17'W)(36°,55'N, 76°,03'W)(36°,52'N, 75°,58'W)(27°,05'N, 97°,23'W)(27°,43'N, 97°,09'W)(27°,50'N, 97°,03'W)(40°,50'N, 72°,30'W)(33°,41'N, 118°,24'W)(38°,36'N, 75°,04'W)(38°,47'N, 75°,09'W)(28°,03'N, 82°,49'W)(30°,17'N, 87°,08'W)(29°,40'N, 85°,23'W)(41°,32'N, 70°,41'W)(43°,00'N, 70°,40'W)(41°,21'N, 70°, 29'W)(41°,32'N, 70°,41'W)(41°,40'N, 70°,00'W)(41°,31'N, 70°,40'W)(42°,42'N, 70°,46'W)(42°,50'N, 70°,48'W)(41°,33'N, 70°,40'W)(43°,00'N, 70°,40'W)(42°,11'N, 70°,42'W)(41°,35'N, 70°,39'W)(43°,32'N, 70°,20'W)(34°,41'N, 76°,40'W)(42°,53'N, 70°,49'W)(42°,53'N, 70°,49'W)(42°53'43" N, 70°49'13" W)(43°00'N, 70°40'W)(41°40'N, 70°00'W)(41°30'N, 70°40'W)(41°20'N, 70°30'W)(42°40N, 70°50'W)(40°50'N, 72°30'W)(41°31' N, 70°40'W)(41°32' N, 70°41 W)(41°32' N, 70°41' W)(41°33' N, 70°40' W)(41°35' N, 70°39' W)(43°00'N, 70°40'W)(41°40'N, 70°00'W)(25°40'N, 80°10'W)(25°30'N, 80°10'W)(24°40'N, 81°20'W)(18°20'N, 64°50'W)(41°31' N, 70°40'W)	(Hummon, 1969, 1974a, 2010; McGeary, 1974; Todaro, Fleeger & Hummon, 1995); Present study.
Wales	(52°,51'N, 04°,34'W)(51°,32'N, 04°,04'W)(52°,28'N, 03°,56'W)	(Hummon, 2010)

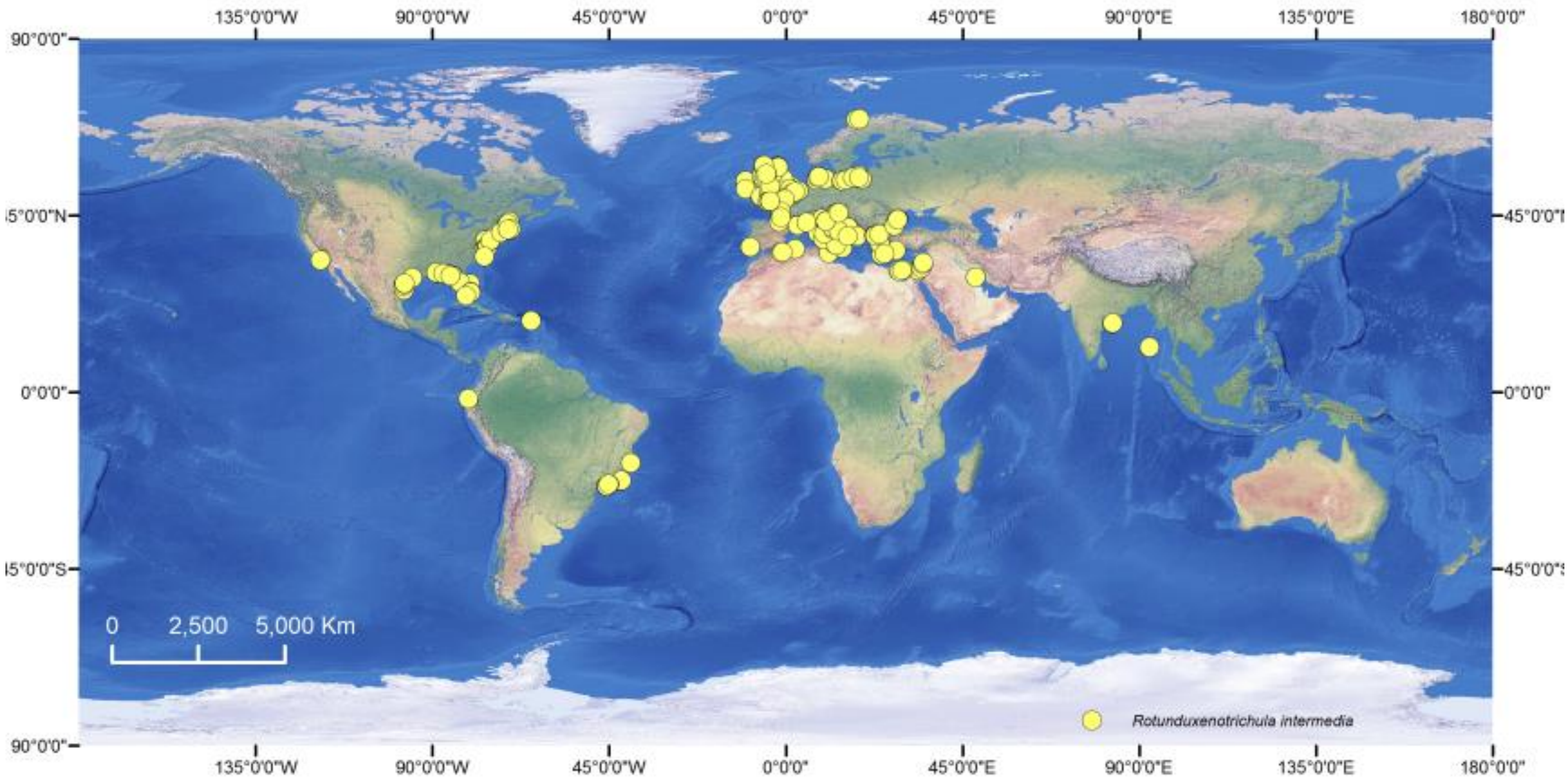

Figure 21 - Map of distribution of *R. intermedia*

Rotunduxenotrichula quadritubulata (Kisielewski, 1988) **nov. comb.**

Syn. *Xenotrichula quadritubulata* Kisielewski, 1988: 189, fig. 1.

Species with body up to 125 µm, subterminal mouth, pharynx length up to 40 µm. Rounded head. Neck weakly marked. Dorsal body covered by 11 rows of hexagonal scales supported by two pedunculumns. Hydrofoil scales are present at ventrolateral side of body. Furcal base covered by flat scales. Two adhesive tubes per side on the furca. Ventral body naked. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn, One pair of locomotory cirri tufts at middle trunk. Paired testes. Rectangular X-organs (Fig, 22A).

Marine Realms: Temperate Northern Atlantic (Fig. 24)

Table 20 - Geographic coordinates of *Rotunduxenotrichula quadritubulata*

Country	Coordinates	Authors
France	(48°43'23.1"N; 3°57'54.2"W)	(Kisielewski, 1988; Hummon, 2010)

Rotunduxenotrichula paralineata (Hummon & Todaro, 2007) **nov. comb.**

Xenotrichula paralineata Hummon and Todaro, 2007: 298, Figs. 1, 3-4.

Species with body up to 185 µm long, terminal mouth, pharynx length 51 µm. Neck weakly marked. Three pairs of sensorial cirri on the head. Oval patch of 14 three-edge flat plates on the central area of the head. Dorsal body covered by four rows of intermedia-type scales on each side of oval patch and 13-16 rows of intermedia-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Hydrofoil scales are present at lateral side of body. Ventral body covered by six longitudinal rows of rhomboidal scales from U55 to the top of furca. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn, One pair of locomotory cirri tufts at middle trunk. Paired testes (Fig. 22B).

Marine Realms: Temperate Northern Atlantic (Fig. 24)

Table 21 - Geographic coordinates of *Rotunduxenotrichula paralineata*

Country	Coordinates	Authors
USA	(30°32’N, 88°56’W)(27°28'N, 97°16'W)(34°38'30"N, 77°05'23"W)(30°,32'N, 88°,55'W)(27°,05'N, 97°,23'W)(34°,38'N, 77°,05'W)(34°38'30"N, 77°05'23"W)	(Ruppert, 1979; Hummon & Todaro, 2007; Hummon, 2010)

Rotunduxenotrichula lineata (Schrom, 1972) **nov. comb.**

Xenotrichula lineata Schrom, 1972: 307, fig. 9

Syn. Xenotrichula lineata - Hummon and Todaro, 2007: 301, Fig. 3

Species with body up to 145 µm long, terminal mouth, pharynx length 48 µm. Neck weakly marked. Oval patch of 16 three-edge flat plates on the central area of the head. Dorsal body covered by four rows of intermedia-type scales on each side of oval patch and 12-16 rows of intermedia-type scales from the neck extending to the top of furca. Furcal base covered by flat scales. Hydrofoil scales are present at lateral side of body. Ventral body naked. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn, One pair of locomotory cirri tufts at middle trunk. Paired testes (Fig. 22C).

Marine Realms: Temperate Northern Atlantic (Fig. 24)

Table 22 - Geographic coordinates of *Rotunduxenotrichula lineata*

Country	Coordinates	Authors
Italy	(45°20'51.7"N , 12°18'55.8"E)(45°21'N, 12°19'E)(45°,37'N, 13°,02'E)(45°,21'N, 12°,19'E)	(Schrom, 1972; Hummon & Todaro, 2007; Hummon, 2010)
USA	(30°32'N, 88°56'W)(26°06'N, 97°09'W)	(Todaro, Fleeger & Hummon, 1995)

Rotunduxenotrichula floridana (Thane-Fenchel, 1970) **nov. comb.**

Xenotrichula floridana Thane-Fenchel, 1970: 126, fig. 9

Species with body up to 250 µm, terminal mouth, pharynx length up to 65 µm. Rounded head. Neck well marked. Dorsal body covered by 12-17 rows of intermedia-type scales to the top of furca. Furcal base covered by flat scales. Ventral body covered by 19 rows of intermedia-type scales to the top of furca. Triangular area of small spines present on the end ventral body. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn, One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 22D).

Marine Realms: Temperate Northern Atlantic (Fig. 24).

Table 23 - Geographic coordinates of *Rotunduxenotrichula floridana*

Country	Coordinates	Authors
USA	(25°43'37.0"N , 80°09'29.6"W)(25°43'37.4"N , 80°08'55.6"W)(25°,43'N, 80°,09'W)(25°,43'N, 80°,09'W)	(Thane-Fenchel, 1970; Ruppert, 1979; Hummon, 2010)

Rotunduxenotrichula soikai (Schrom, 1966) **nov. comb.**

Syn. *Xenotrichula (Xenotrichula) soikai* Schrom, 1966: 37, fig. 3.

Species with body up to 275 µm, terminal mouth, pharynx length up to 54 µm. Rounded head. Neck weakly marked. Dorsal body covered by 12-15 rows of intermedia-type scales to the top of furca. Furcal base covered by flat scales. Ventral body naked. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn, One pair of locomotory cirri tufts at middle trunk. Paired testes are present (Fig. 22E).

Marine Realms: Temperate Northern Atlantic (Fig. 24).

Table 24 - Geographic coordinates of *Rotunduxenotrichula soikai*

Country	Coordinates	Authors
Italy	(45°46'37.6"N , 13°34'24.2"E)(45°,35'N, 12°,52'E)	(Schrom, 1966; Hummon, 2010)

Rotunduxenotrichula micracantha (Remane, 1926) **nov. comb.**

Xenotrichula micracantha (Remane, 1926): 246, fig. 3

Species with body up to 100 µm, terminal mouth, pharynx length up to 25 µm. Rounded head. Neck well marked. Dorsal body covered by 12rows of intermedia-type scales to the top of furca. Furcal base covered by flat scales. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn, One pair of locomotory cirri tufts at middle trunk.

Remarks. R. micracantha is the unique species not included on the phylogenetic analysis due the lack of morphological information described. We assume that this species belong to genus *Rotunduxenotrichula* by the body shape and type of scales (Ruppert, 1979) (Fig. 22F).

Marine Realms: Temperate Northern Atlantic (Fig. 24)

Table 25 - Geographic coordinates of *Rotunduxenotrichula micracantha*

Country	Coordinates	Authors
Germany	(54°10'55.6"N, 7°54'52.0"E)	(Remane, 1926; Hummon, 2010)
Netherlands	(52°,06'N, 04°,12'E)	(Hummon, 2010)
Northern Ireland	(54°,33'N, 05°,33'W)	(Boaden, 1966; Hummon, 2010)

Rotunduxenotrichula carolinensis (Ruppert, 1979) **nov. comb.**

Xenotrichula carolinensis (Ruppert, 1979): 258, figs. 10-12.

Syn. *Xenotrichula carolinensis* ssp. *syltensis* Mock, 1979: 421.

Species with body up to 184 µm, terminal mouth, pharynx length up to 44.7 µm. Rounded head. Neck well marked. Two hard projections on each side of mouth (old Bidentate hypostomium). Three pairs of sensorial cirri on the head. Head with a protuberance on the each anterior lateral margin. Dorsal body covered by 16-20 rows of intermedia-type scales to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly . Furcal base covered by flat scales. Ventral body covered flat scales on the pharynx amd 14 rows of intermedia-type scales from PHJIn to the top of furca. Triangular area of flat scales present on the end ventral body. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present. Paired testes are present (Fig. 22G).

Remarks. We agree with Hummon and Todaro, 2010 who reject synonymizing with *X. intermedia* by Balsamo, Todaro & Tongiorgi, 1992:496; beyond the characters provide by Hummon and Todaro 2010 we add the presence of a pair of protuberances at both sides of anteriormost lateral head. We synonymizing Xenotrichula *carolinensis* ssp. *syltensis* Mock, 1979 with *Rotunduxenotrichula carolinensis* (Ruppert, 1979) by the presence of the crossing of intermedia-type scales on the ventral body at point of the pair of locomotory cirri, however the body length range of Germany species is lower than USA species.

Marine Realms: Temperate Northern Atlantic (Fig. 23)

Table 26 - Geographic coordinates of *Rotunduxenotrichula carolinensis*

Country	Coordinates	Authors
Germany	(55°02'11.6"N , 08°25'38.6"E)(54°54'47.8"N , 08°21'51.4"E)(55°00'17.9"N , 08°23'49.0"E)(54°47'57.7"N, 08°17'41.5"E)(54°57'23.8"N , 08°21'39.0"E)(55°,02'N , 08°,26'E)	(Mock, 1979; Kieneke et al., 2008)
USA	(34°38'30"N, 77°05'23"W)(26°,21'N, 80°,04'W)(29°,42'N, 81°,14'W)(25°,47'N, 80°,07'W)(27°,44'N, 80°,22'W)(30°,07'N, 81°,22'W)(29°,07'N, 83°,03'W)(26°,17'N, 81°,49'W)(27°,29'N, 82°,42'W)(26°,21'N, 81°,53'W)(27°,00'N, 82°,24'W)(27°,05'N, 82°,23'W)(33°,39'N, 78°,55'W)(33°,20'N, 79°,10'W)(29°,12'N, 80°,59'W)(29°,17'N, 80°,59'W)(26°,13'N, 80°,05'W)(30°,01'N, 81°,19'W)(27°,39'N, 80°,21'W)(27°,48'N, 80°,25'W)(29°,53'N, 81°,17'W)(29°,55'N, 81°,17'W)(27°,32'N, 82°,43'W)(27°,28'N, 82°,42'W)(29°,13'N, 85°,24'W)(26°,57'N, 82°,23'W)(26°,44'N, 82°,15'W)(26°,43'N, 82°,15'W)(28°,03'N, 82°,49'W)(27°,38'N, 82°,44'W)(25°,43'N, 80°,09'W)(34°,38'N, 77°,05'W)(33°,20'N, 79°,10'W)(27°35'11.29"N, 80°19'42.55"W)(27°35'26.32"N, 80°19'45.89"W)	(Ruppert, 1979; Hummon, 2010); Present Study

Rotunduxenotrichula punctata (Wilke, 1954) **nov. comb.**

Xenotrichula punctata Wilke, 1954: 519, figs. 13-14.

Species with body up to 180 µm, terminal mouth, pharynx length up to 54 µm. Rounded head. Neck well marked. Dorsal body covered by 17 rows of intermedia-type scales to the top of furca. Hydrofoil scales are present at lateral side of body and increase in size anterior-posteriorly .

Furcal base covered by flat scales. Ventral body covered by of pedunculate scales on the each lateral side and two rows of flat small scales from PHJIn to the top of furca. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 22H).

Marine Realms: Temperate Northern Atlantic, Tropical Eastern Pacific, Tropical Atlantic, Western Indo-Pacific (Fig. 23).

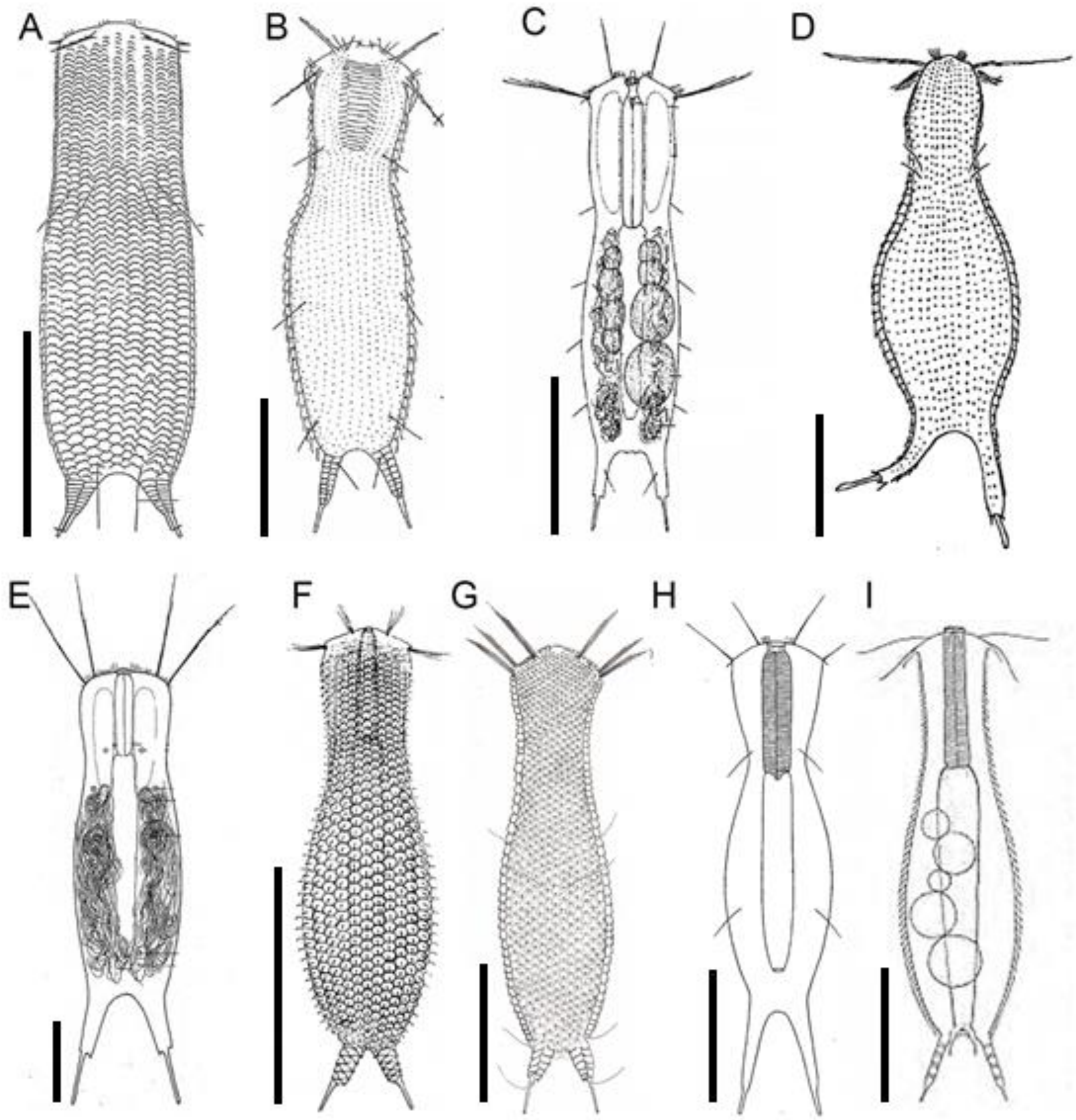

Figure 22 - Schematic draw. A Dorsal body of *R. quadritubulata* (modified from Kisielewski, 1988). B - Dorsal body of *R. paralineata* (modified from Hummon and Todaro, 2007). C – Internal body of *R. lineata* (modified from Schrom, 1972). D - Dorsal body of *R. floridana* (modified from Thane-Fenchel, 1970). E - Internal body of *R. soikai* (modified from Schrom, 1966). F - Dorsal body of *R. micracantha* (modified from Remane, 1926). G - Dorsal body of *R. carolinensis* (modified from Ruppert, 1979). H - Internal body of *R. punctata* (modified from Wilke, 1954). I - Internal body of *R. bispina* (modified from Roszczak, 1939).

Table 27 - Geographic coordinates of *Rotunduxenotrichula punctata*

Country	Coordinates	Authors
Denmark	(55°05'21.7"N, 8°33'54.5"E)	(Mock, 1979)
Egypt	(31°,14'N, 29°,58'E)(31°,08'N, 33°,43'E)(31°,20'N, 27°,14'E)(26°,03'N, 33°,54'E)(27°,10'N, 33°,57'E)(29°,14'N, 32°,53'E)(28°,05'N, 34°,25'E)(27°,56'N, 34°,23'E)(28°,50'N, 34°,36'E)(27°,43'N, 34°,15'E)(29°,52'N, 32°,38'E)(27°,46'N, 34°,11'E)(31°,18'N, 30°,02'E)(27°,13'N, 33°,52'E)(27°,46'N, 34°,12'E)(29°,26'N, 32°,45'E)(29°,25'N, 32°,30'E)(29°,10'N, 32°,57'E)(27°,54'N, 34°,21'E)(27°,15'N, 33°,48'E)(27°,08'N, 33°,49'E)(27°,53'N, 34°,19'E)(25°,54'N, 33°,59'E)(25°,44'N, 33°,57'E)(27°,50'N, 34°,15'E)(27°,50'N, 34°,18'E)(29°,36'N, 32°,40'E)	(Hummon, 2010)
England	(52°,58'N, 00°,29'E)	(Hummon, 2010)
France	(44°39'41.9"N , 1°14'20.6"W)(44°40'26.9"N , 1°14'20.1"W)(44°37'44.0"N , 1°14'34.2"W)(44°39'47.0"N , 1°09'13.1"W)(44°34'03.7"N , 1°14'06.9"W)(45°38'29.5"N , 1°05'14.1"W)(44°,39'N, 01°,15'W)(44°,39'N, 01°,10'W)(44°,42'N, 01°,15'W)(44°,38'N, 01°,15'W)(44°,33'N, 01°,17'W)(44°,42'N, 01°,15'W)(44°,38'N, 01°,13'W)(44°,39'N, 01°,15'W)(43°,11'N, 05°,41'E)(42°,44'N, 09°,20'E)(2°,50'N, 09°,24'E)(42°,06'N, 08°,41'E)	(D'Hondt, 1966, 1968a; Hummon, 2010)
Germany	(55°02'11.6"N , 8°25'38.6"E)(54°54'47.8"N , 8°21'51.4"E)(55°03'27.4"N , 8°25'25.4"E)(55°,03'N, 08°,26'E)(54°,54'N, 08°,22'E)(55°,02'N , 08°,26'E)	(Mock, 1979; Hummon, 2010)
Greece	(37°,32'N, 22°,47'E)(36°,05'N, 27°,45'E)(35°,14'N, 26°,16'E)(36°,21'N, 27°,57'E)(35°,05'N, 26°,14'E)	(Hummon, 2010)
Isle Of Man	(54°,06'N, 04°,39'W)	(Hummon, 2010)
Israel	(29°,30'N, 34°,55'E)(29°,29'N, 34°,54'E)	(Hummon, 2010)
Italy	(39°,07'N, 08°,21'E)(38°,58'N, 08°,26'E)(40°,37'N, 09°,46'E)(40°,26'N, 09°,50'E)(45°46'37.6"N , 13°34'24.2"E)(40°,50'N, 14°,15'E)(40°,49'N, 14°,13'E)(40°,49'N, 14°,12'E)(40°47'N, 13°26'E)(41°13'N, 13°05'E)(41°13'N, 09°17'E)(40°35′N, 08°15′E)(45°,46'N, 13°,09'E)(44°,49'N, 12°,17'E)(39°,07'N, 08°,21'E)(40°,49'N, 14°,13'E)(41°,13'N, 09°,17'E)(40°,44'N, 13°,55'E)(40°,50'N, 14°,06'E)(40°,47'N, 14°,12'E)(40°,45'N, 13°,53'E)(41°,13'N, 13°,05'E)(40°,49'N, 14°,12'E)(38°,58'N, 08°,26'E)(40°,50'N, 14°,15'E)(40°,58'N, 17°,18'E)(45°,45'N, 13°,31'E)(41°,51'N, 16°,11'E)(40°,22'N, 18°,18'E)(37°,45'N, 12°,28'E)(42°,49'N, 10°,44'E)(41°,03'N, 13°,53'E)(40°,37'N, 09°,46'E)(40°,50'N, 14°,15'E)(40°,50'N, 14°,01'E)(40°,45'N, 13°,56'E)(40°,45'N, 13°,56'E)(40°,26'N, 09°,50'E)(,)(40°,47'N, 13°,36'E)	(Wilke, 1954; Balsamo, Fregni & Tongiorgi, 1995; Todaro et al., 2003; Leasi & Todaro, 2008; Hummon, 2010)
Norway	(60°,17'N, 05°,09'E)	(Hummon, 2010)
Scotland	(54°,48'N, 04°,56'W)	(Hummon, 2010)
Sweden	(58º15'07"N, 11º27'57"E	(Hummon, 2010)
USA	(38°,47'N, 75°,09'W)(25°,47'N, 80°,07'W)(29°,17'N, 80°,59'W)(27°,32'N, 82°,43'W)(29°,13'N, 85°,24'W)(26°,44'N, 82°,15'W)(26°,43'N, 82°,15'W)(29°,40'N, 85°,23'W)(24°,39'N, 81°,15'W)(25°,43'N, 80°,09'W)(25°,31'N, 80°,11'W)(41°,32'N, 70°,41'W)(41°,31'N, 70°,40'W)(43°,00'N, 70°,40'W	(Hummon, 2010)
Virgin Islands	(18°,21'N, 64°,52'W)(18°,20'N, 64°,51'W)	(Hummon, 2010)
Wales	(51°,31'N, 04°,03'W)	(Hummon, 2010)

Rotunduxenotrichula bispina (Roszczak, 1939) **nov. comb.**

Xenotrichula bispina Roszczak, 1939:10, fig. 4

Species with body up to 235 µm, terminal mouth, pharynx length up to 50 µm. Rounded head. Head with small flat scales on the dorsal region. Neck well marked. Two hard projections on each side of mouth (old Bidentate hypostomium). Three pairs of sensorial cirri on the head. Dorsal body covered by intermedia-type scales to the top of furca.. Furcal base covered by flat scales. Ventral body covered by intermedia-type scales on each lateral side of intestine from U49 to the top of furca. Three pairs of spines on the intrafurcal region. Locomotory cirri with equal sizes, two longitudinal bands extending from the head to the PHJIn. One pair of locomotory cirri tufts at middle trunk. Paired testes and ovaries are present (Fig. 22I).

Marine Realms: Temperate Northern Atlantic (Fig. 23)

Table 28 - Geographic coordinates of *Rotunduxenotrichula bispina*

Country	Coordinates	Authors
France	(43°39'48.5"N , 1°25'52.9"W)	(D'Hondt, 1968a)
Germany	(55°02'11.6"N , 8°25'38.6"E)(55°,02'N , 08°,26'E)	(Mock, 1979; Hummon, 2010)
Poland	(54°50'07.8"N , 18°18'38.7"E)(54°45'36.3"N , 18°30'46.6"E)(54°44'29.0"N , 18°33'56.2"E)(54°49' N, 18°21' E)(54°50' N, 18°18' E)(54°49' N, 18°21' E)(54°45' N, 18°30' E)(54°44' N, 18°34')(54°45' N, 18°30' E)(54°,55'N, 18°,21'E)(54°44'57.7"N , 18°40'09.7"E)	(Roszczak, 1939; Hummon, 2010; KOLICKA et al., 2014)
Romania	(43°,49'N, 28°,33'E)	(Rudescu, 1966; Hummon, 2010)

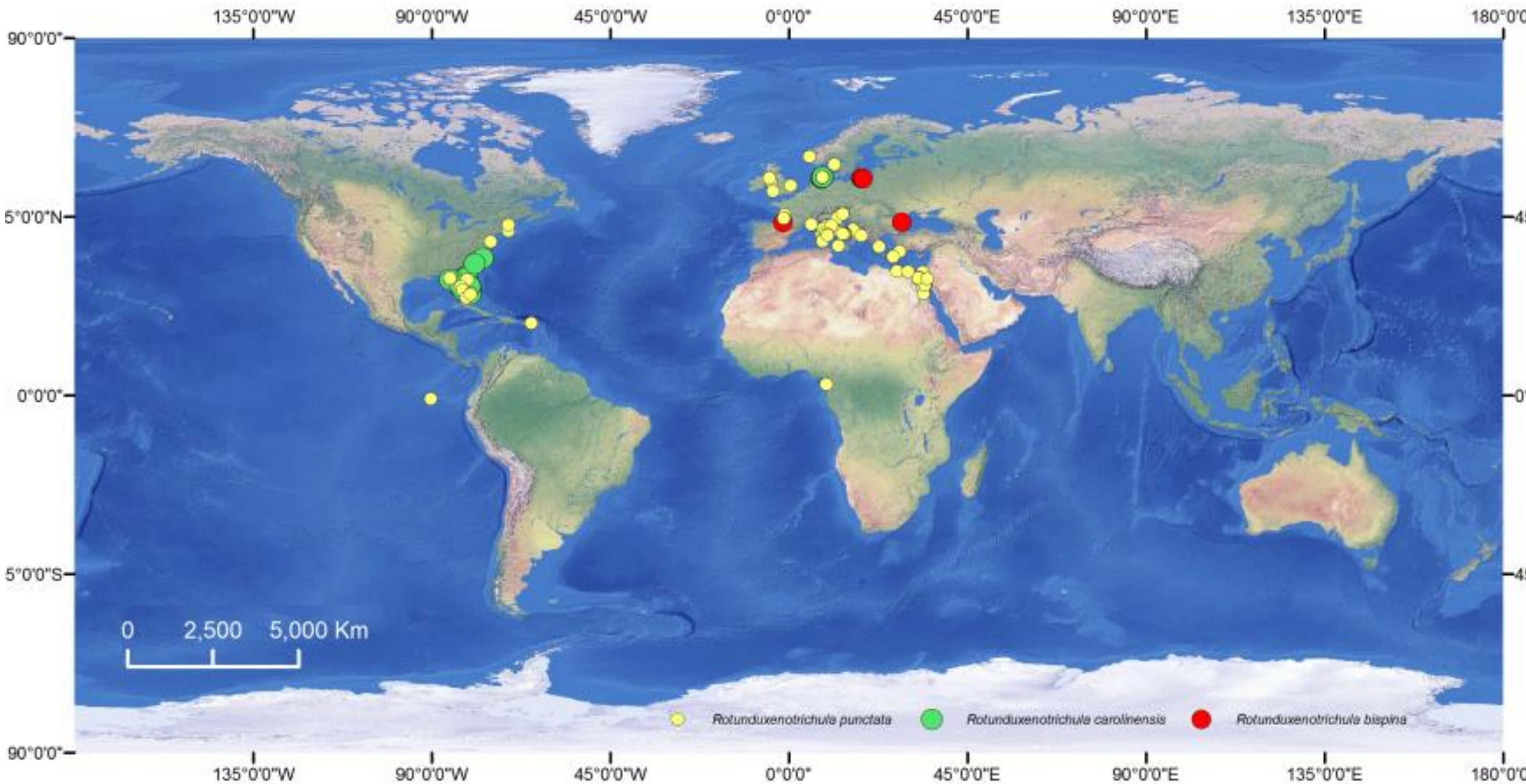

Figure 23 - Map of distribution of *R. punctata*, *R. carolinensis* and *R. bispina*.

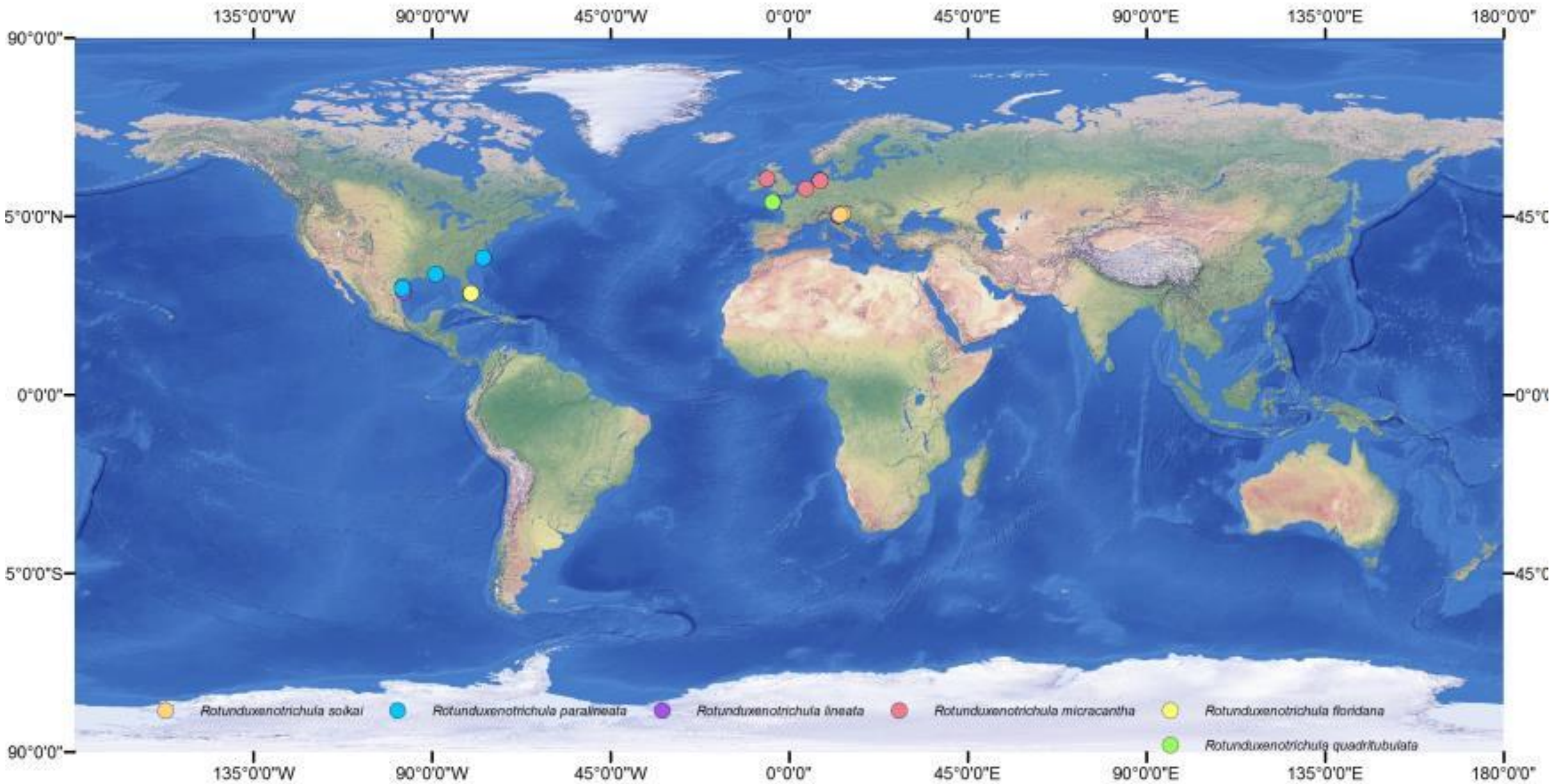

Figure 24 - Map of distribution of *R. soikai, R. paralineata, R. lineata, R. micracantha, R. floridana* and *R. quadritubulata.*

Identification key
Xenotrichulidae: Subfamilies

1	Xenotrichulidae with elongated body; head with a set of plates in dorsal, ventral and lateral region; ventral cirri arranged in two lateral longitudinal rows from the head to the middle body. ………………………………….Draculiciterinae, *Draculiciteria tesselata*
1'	Xenotrichulidae with tenpin-like body; head weakly marked; furca distinctly delimited from the trunk; rounded head………………………………………..2
2	Cephalion absent; intermedia-type or hexagonal scales ……..……………..… Rotundunxenotrichulinae
2	Long or short tentacle on the lateral head; ; cephalion present; locomotory cirri with different sizes…………….Xenotrichulinae

Xenotrichulidae: Rotundunxenotrichulinae: genus and species.

1	Sensorial bristles on the dorsal body present………………………. 2
1'	Dorsal body covered by 12-15 rows of intermedia-type scales; ventral body naked; dorsal sensorial bristles absent ………… *Rotundunxenotrichula soikai*
2	One pair of sensorial bristles on the dorsal neck present…………………….. 3
2'	Two pairs of sensorial bristles on the dorsal neck present……………………. 7
3	Three pairs of sensorial cephalic tufts, pair of hard projections on each side of mouth present……………………….……………………………………4
3'	Two pairs of sensorial cephalic tufts; Oval patch of 16 three-edge flat plates on the central area of the head……………………. *Rotundunxenotrichula lineata*
4	Ventral scales supported by pedunculum……………………………………..5
4'	Oval patch of 14 three-edge flat plates on the central area of the head; ventral body covered by six longitudinal rows of rhomboidal scales from U55…….. *Rotundunxenotrichula paralineata*
5	Hydrofoil scales on ventrolateral body; triangular area of flat scales on the ventral end body ....…………………….6
5'	Pedunculate scales on ventrolateralbody; three pairs of spines on the intrafurcal

	region………………………………………….. *Rotundunxenotrichula bispina*
6	Ventral body covered by nine rows of intermedia-type ……………. *Rotundunxenotrichula intermedia*
6’	Head with a protuberance on the each anterior lateral margin; Ventral pharynx covered by flat scales; Lateral part of ventral trunk covered by intermedia-type scales; presence of the crossing of intermedia-type scales at point of the pair of locomotory cirri tufts……………………… *Rotundunxenotrichula carolinenses*
7	One adhesive tube on each furca…………………………………………..8
7’	Dorsal body covered by 11 rows of hexagonal scales; two pedunculums supporting scales; two adhesive tubes on each furca…….. *Rotundunxenotrichula quadritubulata*
8	Ventral body covered by 19 rows of intermedia-type scales; triangular area of small spines present on the end ventral body……… *Rotundunxenotrichula floridana*
8’	Lateral part of ventral trunk covered by intermedia-type scales; ventral field covered by two rows of flat small scales from PHJIn to the top of furca………. *Rotundunxenotrichula punctata*

Xenotrichulidae: Xenotrichulinae: genera.

1	Long tentacle; cephalion region covered by scales; dorsal body covered by minute elliptical scales; hydrofoil scales absent; ventral body without scales; anterior ventral head covered by cirri and divided in two longitudinal rows extending to the PHJIn…………………..………………*Minutuxenotrichula*
1’	Long or short tentacle; Cephalion region covered by plate or scales or uncovered; oval patch of transversal flat plates can be present on the dorsal head; transversal row of cilia on each side of mouth extending to total ventral length of tentacle; subterranea-type or flat oval scales present; Hydrofoil scales usually present; ventral body covered by scales; longitudinal plates on the middle trunk to the end………………………………………………………….*Xenotrichula*

Xenotrichulidae: Xenotrichulinae: *Minutuxenotrichula*: species

1	Transversal row of cilia on each side of mouth extending to total ventral length of tentacle; one pair of locomotory cirri tufts at middle trunk…………………………………………. *Minutuxenotrichula tentaculata*
1’	One pair of locomotory cirri tufts at middle trunk and one pair on the posterior gut ……..……………………………………. *Minutuxenotrichula laccadivensis*

Xenotrichulidae: Xenotrichulinae: *Xenotrichula*: species

1	Long tentacles…………………………………………………………………………2
1’	Short tentacles……………………………………………..……………………..5
2	Six longitudinal rows of flat large plates at ventral trunk side; inners rows presents 20 plates……………………..………………………………. *Xenotrichula guadelupensis*
2’	Scales increasing in size along body length ……………………..……………3
3	Eight longitudinal rows of flat plates at ventral trunk side; Inner and outer rows are small in size; Ventral cirri equal size.……………………………. *Xenotrichula cornuta*
3’	Sensorial bristles on the neck and trunk …………………………………………………4
4	Scales and bristles covering all tentacle length; sensorial bristle on dorsal head; Four rows of plate at posterior part of the trunk; inners rows present 20 plates……………….. *Xenotrichula velox*
4’	Imbricate small cephalion; double edge scales on the ventral pharynx; two rows of 21 plates at posterior part of the trunk; 17 small plates between large plates and hydrofoil scales on each side of body…………………………………… *Xenotrichula tropicalis*
5	Dorsal body covered by oval flat scales………………….…..…………..…………..6
5’	Oval patch of tranversal flat plates on the central area of the dorsal head; dorsal body covered by pedunculate scales………..…………..………..………..………….. 9
6	Sensorial bristle on the dorsal head; sensorial bristle on anterior third of dorsal trunk; Two pairs of sensorial bristles on base of furca...………………………………..……………….. 7
6’	Absence of sensorial bristle on the dorsal head ……………….…………….……………8
7	Five pairs of dorsal sensorial bristles; Ventral body covered with seven rows of oval scales ……………………………………………………….*Xenotrichula squamosa*

7'	Three pairs of dorsal sensorial bristles; oral bristles not present; *Xenotrichula pygmaea*
8	Ventral body covered oval scales; Hydrofoil scales present; sensorial bristle only on the base of furca…………………………………………….*Xenotrichula arcassonensis*
8'	Two rows of oval scales on each lateral side of ventral body; Four rows of flat plates at ventral trunk; three pairs of dorsal sensorial bristles……………..*Xenotrichula wilkeae*
9	Cephalion region uncovered………………………………………………………… 10
9'	Cephalion region covered by small plate; dorsal body covered by 17 rows of subterranea-type scales; ventral body covered by pedunculated subterranea-type scales from the U48 to the top of furca…………………………………… *Xenotrichula texana*
10	Oval patch of tranversal flat plates possessing three edges………………………11
10'	Oval patch of tranversal flat plates without edge; ventral body covered by pedunculated subterranea-type scales from the U48 to the top of furca. Two longitudinal rows of flat plates ventral trunk side………………………………..…….. *Xenotrichula simplex*
11	Sensorial head tufts inserted on the dorsal and lateral side of head…………….12
11'	Oval patch of 15 flat plates on the central area of the head; ventral body covered five rows of subterranea-type scales on each lateral body from U51 to the top of furca; Three rows of small plates at middle ventral of the trunk. ……..…….. *Xenotrichula affinis*
12	Ventral body without scales; flat plates covering from U51 to the top of furca……… *Xenotrichula subterranea*
12'	Ventral body covered by six rows of pedunculate scales from the U48 to the top of furca; two pairs of dorsal sensorial bristle on the end of trunk…………… *Xenotrichula transatlantica.*

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References

Abukawa, S. & Kajihara, H. 2011. Comments on the nomenclatural status of two subgeneric names for marine gastrotrichs (Gastrotricha: Xenotrichulidae). Zootaxa 2732: 68.

Araújo TQ., Atherton S., Hochberg R. 2015. First record and a description of a new species of *Oregodasys* (Gastrotricha: Macrodasyida: Thaumastodermatidae) from Tobago. *Proceedings of the Biological Society of Washington* 128:190–199. DOI: 10.2988/0006-324X-128.3.176.

Balsamo M., D`Hondt J-L., Kisielewski J., Todaro M., Tongiorgi P., Guidi L., Grilli P., de Jong Y. 2015. Fauna Europaea: Gastrotricha. *Biodiversity Data Journal* 3:e5800. DOI: 10.3897/BDJ.3.e5800.

Balsamo M., Fregni E., Tongiorgi P. 1995. Marine Gastrotricha from the coasts of Sardinia (Italy). *Bolletino di Zoologia* 62:273–286. DOI: 10.1080/11250009509356076.

Balsamo M., Fregni E., Tongiorgi P. 1996. Marine Gastrotricha from Sicily with the description of a new species of Chaetonotus. *Italian Journal of Zoology* 63:173–183. DOI: 10.1080/11250009609356127.

Balsamo M., Guidi L., D'Hondt JL. 2013. Phylum Gastrotricha. *Zootaxa* 3703:79–82. DOI: 10.11646/zootaxa.3703.1.16.

Balsamo M., Todaro MA. 2002. Gastrotricha. In: Rundle SD, Robertson AL, Schmid-Araya JM eds. *Freshwater Meiofauna: Biology and Ecology*. Leiden: Backhuys Publishers, 45–61.

Balsamo M., Todaro MA., Tongiorgi P. 1992. Marine gastrotrichs from the Tuscan Archipelago (Tyrrhenian Sea): II. Chaetonotida, with description of three new species. *Bolletino di zoologia* 59:487–498.

Bekkouche N., Worsaae K. 2016. Neuromuscular study of early branching *Diuronotus aspetos* (Paucitubulatina) yields insights into the evolution of organs systems in Gastrotricha. *Zoological Letters* 2:21. DOI: 10.1186/s40851-016-0054-3.

Boaden PJS. 1963. Marine Gastrotricha from the Interstitial Fauna. *Proceedings of the Zoological Society of London* 140:485–502. DOI: 10.1111/j.1469-7998.1963.tb01869.x.

Boaden PJS. 1966. Interstitial Fauna from Northern Ireland. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* 2:125–130.

D'Hondt JL. 1966. Chaetonotoides marins et d'eau douce de la région d'Arcachon. *Actes de la Société Linnéenne de Bordeaux* 103A:1–22.

D'Hondt JL. 1968a. Contribution à la connaissance des Gastrotriches intercôtidaux du Golfe de Gascogne. *Cahiers de Biologie Marine* 9:387–404.

D'Hondt JL. 1968b. Gastrotriches et Halammohydrides des côtes Flammandes et Picardes. *Bulletin du Muséum National d'Histoire Naturelle* 40:214–227.

D'Hondt JL. 1970. Inventaire de la faune marine de Roscoff (gastrotriches, kinorynques, rotifere, tardigrades). *Travaux de la Station Biologique de Roscoff NS* 16:1–29.

D`Hondt J-L., Balsamo M. 2009. Remarques systématiques èur quelques espèces de Gastrotriches marins. *Bulletin de la Société linnéenne de Bordeaux* 144:263–279.

Delamare-Deboutteville C. 1954. Eaux souterraines littorales de la côte Catalane française (Mise au point faunistique). *Vie et Milieu* 5:408–451.

Forey PL., Kitching I. 2000. Experiments in coding multistate characters. In: Scotland R, Pennington T eds. *Homology and systematics: coding characters for phylogenetic analysis*. London: Taylor & Francis, 54–80.

Forneris L. 1961. Beiträege zur Gastrotrichenfauna der Nord- und Ostsee. *Kieler Meeresforschungen* 17:206–218.

Garraffoni ARS., Araújo TQ., Lourenço AP., Guidi L., Balsamo M. 2016. A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. *Systematics and Biodiversity*. DOI: 10.1080/14772000.2016.1214189.

Garraffoni ARS., Di Domenico M., Hochberg R. 2016. New records of marine Gastrotricha from São Sebastião Island (Brazil) and the description of a new species. *Marine Biodiversity*:1–9. DOI: 10.1007/s12526-016-0486-1.

Gerlach SA. 1953. Gastrotrichen aus dem Küestengrundwasser des Mittelmeeres. *Zoologischer Anzeiger* 150:203–211.

Goloboff PA., Farris JS., Nixon KC. 2003. T.N.T.: Tree Analysis Using New Technology.

Goloboff PA., Farris JS., Nixon KC. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24:774–786. DOI: 10.1111/j.1096-0031.2008.00217.x.

Gosse PH. 1864. The natural history of the hairy-backed animalcules (Chaetonotidae). *The Intellect. Observer* 5:387–406.

Hawkins J a., Hughes CE., Scotland RW. 1997. Primary Homology Assessment, Characters and Character States. *Cladistics* 13:275–283. DOI: 10.1111/j.1096-0031.1997.tb00320.x.

Hennig W. 1966. *Phylogenetic Systematics*. Urbana: University of Illinois Press.

Hochberg R., Litvaitis MK. 2001. The musculature of Draculiciteria tessalata (Chaetonotida, Paucitubulatina): implications for the evolution of dorsoventralmuscles in Gastrotricha. *Hydrobiologia* 452:155–161. DOI: 10.1023/A:1011940430507.

Hummon WD. 1969. Distributional ecology of marine interstitial Gastrotricha from Woods Hole, Massachusetts, with taxonomic comments on previously described species. University of Mas5achusetts.

Hummon WD. 1974a. S H? : A similarity index based on shared species diversity, used to assess temporal and spatial relations among intertidal marine Gastrotricha. *Oecologia* 17:203–220. DOI: 10.1007/BF00344922.

Hummon WD. 1974b. Some taxonomic revisions and nomenclatural notes concerning marine and brackish-water gastrotricha. *Transactions of the American Microscopical Society* 93:194–205.

Hummon WD. 1975. Seasonal changes in secondary production, fuanal similarity and biological accommodation, related to stability among the Gastrotricha of two semi-enclosed Scottish beaches. *10th European Symposium on Marine Biology* 2:309–336.

Hummon WD. 2008. Brackish-water Gastrotricha of the Polish Baltic Coast. *Meiofauna Marina* 16:109–116.

Hummon WD. 2010.Global distribution of marine Gastrotricha. *Available at http://www.gastrotricha.unimore.it/checklist.htm:Global_distribution_of_marine_Gastrotricha.pdf]*

Hummon WD., Todaro MA. 2007. A new species of Xenotrichulidae (Gastrotricha) from southern and southeastern USA. *Cahiers de Biologie Marine* 48:297–302.

Hummon WD., Todaro MA. 2010. Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. *Zootaxa* 32:1–32.

Hummon WD., Warwick RM. 1990. The marine flora and fauna of the Isles of Scilly—Gastrotricha. *Journal of Natural History* 24:519–525. DOI: 10.1080/00222939000770361.

Jansson B-O. 1968. Quantitative and experimental studies of the interstitial fauna in four Swedish sandy beaches. *Ophelia* 5:1–71. DOI: 10.1080/00785326.1968.10409625.

Jouk PEH., Hummon WD., Hummon MR., Roidou E. 1992. Marine Gastrotricha from the Belgian coast: species list and distribution. *Bulletin van het Koninklijk Belgisch Instituut voor Natuurwetenschappen, Biologie* 62:87–90.

Kånneby T. 2016. Phylum Gastrotricha. In: *Thorp and Covich's Freshwater Invertebrates*. Elsevier, 115–130. DOI: 10.1016/B978-0-12-385028-7.00007-X.

Kånneby T., Todaro MA., Jondelius U. 2012. A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. *Hydrobiologia* 683:185–202. DOI: 10.1007/s10750-011-0956-1.

Kånneby T., Todaro MA., Jondelius U. 2013. Phylogeny of Chaetonotidae and other Paucitubulatina (Gastrotricha: Chaetonotida) and the colonization of aquatic ecosystems. *Zoologica Scripta* 42:88–105. DOI: 10.1111/j.1463-6409.2012.00558.x.

Kaplan G. 1958. Premières observations sur les gastrotriches psammophiles des côtes du Calvados. *Archive de Zoologie Expérimentale et Générale* 1:27–37.

Karling TG. 1954. Über einige Kleintiere des Meeressandes des Nordsee-Ostsee-Gebietes. *Arkiv foer zoologi* 7:241–249.

Kieneke A., Ahlrichs WH., Martínez Arbizu P., Bartolomaeus T. 2008. Ultrastructure of protonephridia in *Xenotrichula carolinensis syltensis* and *Chaetonotus maximus* (Gastrotricha: Chaetonotida): comparative evaluation of the gastrotrich excretory organs. *Zoomorphology* 127:1–20. DOI: 10.1007/s00435-007-0051-3.

Kieneke A., Riemann O., Ahlrichs WH. 2008. Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. *Zoologica Scripta* 37:429–460. DOI: 10.1111/j.1463-6409.2008.00334.x.

Kieneke A., Schmidt-Rhaesa A., Hochberg R. 2015. A new species of *Cephalodasys* (Gastrotricha, Macrodasyida) from the Caribbean Sea with a determination key to species of the genus. *Zootaxa* 3947:367. DOI: 10.11646/zootaxa.3947.3.4.

Kisielewski J. 1975. Brzuchorzeski (Gastotricha) psammonowe Polskiego Baltyku. *Badania Fizjograficzne nad Polska Zachodnia* 28C:7–40.

Kisielewski J. 1984. *Xenotrichula guadelupense* n.sp., a new marine gastrotrich from Guadeloupe. *Bulletin du Muséum National d'Histoire Naturelle Paris* 6A:37–40.

Kisielewski J. 1988. New records of marine gastrotricha from the French coasts of Manche and Atlantic .2. Chaetonotida, with description of 4 new species. *Cahiers De Biologie Marine* 29:187–214.

Kolicka M. 2017. New Arctic Gastrotricha — Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici* 67:131–180. DOI: 10.3161/00034541ANZ2017.67.1.015.

Kolicka M., Dabert M., Dabert J., Kånneby T., Kisielewski J. 2016. *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics* 30:398. DOI: 10.1071/IS16001.

Kolicka M., Janikowska E., Kotwicki L. 2015. Baltic Sea Gastrotricha—one new species and one new record of Chaetonotida from Poland. *Zootaxa* 4027:487. DOI: 10.11646/zootaxa.4027.4.2.

Kolicka M., Kisielewski J., Kotwicki L., Zawierucha K., Grzelak K. 2014. Checklist of Gastrotricha of the Polish Baltic Sea with the first reports of *Heterolepidoderma joermungandri* Kånneby, 2011, and *Turbanella hyalina* Schultze, 1853. *Zootaxa* 3869:101. DOI: 10.11646/zootaxa.3869.2.1.

Leasi F., Todaro MA. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). *Biological Journal of the Linnean Society* 94:379–398. DOI: 10.1111/j.1095-8312.2008.00974.x.

Leasi F., Todaro MA. 2009. Meiofaunal cryptic species revealed by confocal microscopy: the case of *Xenotrichula intermedia* (Gastrotricha). *Marine Biology* 156:1335–1346. DOI: 10.1007/s00227-009-1175-4.

Levi C. 1950. Contributions à l'étude des Gastrotriches de la région de Roscoff. *Archive de Zoologie Expérimentale et Générale* 87:30–42.

Luporini P., Magagnini G., Tongiorgi P. 1971. Contribution a la connaissance desgastrotriches des cotes de Toscane. *Cahiers de Biologie Marine* 12:435–455.

Luporini P., Magagnini G., Tongiorgi P. 1973. Chaetonotoid Gastrotrichs of the Tuscan Coast. *Bolletino di zoologia* 40:31–40. DOI: 10.1080/11250007309427479.

McGeary KR. 1974. The biology of marine Gastrotricha from Hampton Harbor, New Hampshire. University of New Hampshire.

Mock H. 1979. Chaetonotoidea (Gastrotricha) der Nordseeinsel Sylt. *Mikrofauna des Meeresbodens* 78:403–507.

Naidu K V., Rao GC. 2004. *Fauna of India and the Adjacent Countries : Gastrotricha*. Kolkata: Zoological Survey of India.

Páll-Gergely B. 2017. Should we describe genera without molecular phylogenies? *Zootaxa* 4232:593. DOI: 10.11646/zootaxa.4232.4.11.

Paps J., Riutort M. 2012. Molecular phylogeny of the phylum Gastrotricha: New data brings together molecules and morphology. *Molecular Phylogenetics and Evolution* 63:208–212. DOI: 10.1016/j.ympev.2011.12.010.

Pleijel F. 1995. On character coding for phylogeny reconstruction. *Cladistics* 11:309–315. DOI: 10.1016/0748-3007(95)90018-7.

Rao GC. 1969. The marine interstitial fauna inhabiting the beach sands of Orissa Coast. *Journal of the Zoological Society of India* 21:89–104.

Rao GC. 1989. Intertidal meiofauna. In: *Fauna of Orissa. Part 2.* 1–77.

Rao GC. 1991. *Meiofauna*. Calcutta: Zoological Survey of India.

Rao GC., Clausen C. 1970. *Planodasys marginalis* gen. et sp. nov. and Planodasyidae fam. nov. (Gastotricha Magrodasyoidea). *Sarsia* 42:73–82. DOI: 10.1080/00364827.1970.10411164.

Rao GC., Ganapati PN. 1967. On some interstitial polychaetes from the beach sands of Waltair Coast. *Proceedings of the Indian Academy of Sciences - Section B* 66:10–15. DOI: 10.1007/BF03052406.

Rao GC., Ganapati PN. 1968. Some new interstitial gastrotrichs from the beach sands of Waltair coast. *Proceedings of the Indian Academy of Sciences - Section B* 67:35–53.

Remane A. 1925. Organisation und systematische Stellung der aberranten Gastrotrichen. *Zoologischer Anzeiger* 1:121–128.

Remane A. 1926. Marine Gastrotrichen aus der Ordung der Chaetonotoidea. *Zoologischer Anzeiger* 66:243–252.

Remane A. 1927. *Xenotrichula velox* nov. gen. nov. spec., ein chaetonotoides Gastrotrich mit mäennlichen Geschlectsorganen. *Zoologischer Anzeiger* 71:289–294.

Remane A. 1934. Die Gastrotrichen des Küestengrundwassers von Schilksee. *Schr. Naturwiss. Ver. Schleswig-Holstein* 20:473–478.

Remane A. 1936. Gastrotricha. In: Bronn HG ed. *Klassen und Ordnungen des Tierreichs*.

Leipzig: Akademische Verlagsgesellschaft, 1–242.

Renaud-Mornant J. 1968. Présence du genre Polymerurus en milieu marin. Description de deux espèces nouvelles (Gastrotricha, Chaetonotoidea). *Pubblicazioni della station zooogica di Napoli* 36:141–151.

Roszczak R. 1939. Die Psammitgastrotricha des polnischen Ostseestrandes. *Zool. Poloniae* 4:1–24.

Rudescu L. 1966. Gastrotrichi voi pentra Marea Neagra (Sistematica, ecologia si zoogeografia grupului). *Hydrobiologia* 7:103–130.

Ruppert EE. 1979. Morphology and systematics of the Xenotrichulidae (Gastrotricha, Chaetonotida). *Mikrofauna des Meeresbodens* 76:239–292.

Schmidt P. 1974. Interstitielle fauna von Galapagos - 4. Gastrotricha. *Mikrofauna des Meeresbodens* 26:497–570.

Schrom H. 1966. Gastrotrichen aus Feinsanden der Umgebung von Venedig. *Bollettino del Museo Civico di Storia Naturale di Venezia* 17:31–45.

Schrom H. 1972. Nordadriatische Gastrotrichen. *Helgoländer Wissenschaftliche Meeresuntersuchungen* 23:286–351. DOI: 10.1007/BF01609680.

Spalding MD., Fox HE., Allen GR., Davidson N., Ferdaña ZA., Finlayson M., Halpern BS., Jorge MA., Lombana A., Lourie SA., Martin KD., Mcmanus E., Molnar J., Recchia CA., Robertson J. 2007. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience* 57:573. DOI: 10.1641/B570707.

Swedmark B. 1956. Etude de la microfaune des sables marins de la region de Marseille. *Archives de Zoologie Expérimentale et Générale* 93:70–95.

Thane-Fenchel A. 1970. Interstitial gastrotrichs in some south Florida beaches. *Ophelia* 7:113–138. DOI: 10.1080/00785236.1970.10419293.

Todar., Balsamo M., Kristensen RM. 2005. A new genus of marine chaetonotids (Gastrotricha), with a description of two new species from Greenland and Denmark.

Todaro MA. 1992. Contribution to the study of the Mediterranean meiofauna: Gastrotricha from the Island of Ponza, Italy. *Bolletino di zoologia* 59:321–333. DOI: 10.1080/11250009209386689.

Todaro MA. 1994. Chaetonotus triacanthus and Heteroxenotrichula texana, Two New Chaetonotid Gastrotrichs from the Gulf of Mexico. *Transactions of the American*

Microscopical Society 113(1):15–21.

Todaro M., Dal Zotto M., Bownes S., Perissinotto R. 2011. First records of Gastrotricha from South Africa, with description of a new species of Halichaetonotus (Chaetonotida, Chaetonotidae). *ZooKeys* 142:1–13. DOI: 10.3897/zookeys.142.2036.

Todaro MA., Fleeger JW., Hu YP., Hrincevich AW., Foltz DW. 1996. Are meiofaunal species cosmopolitan? Morphological and molecular analysis of Xenotrichula intermedia (Gastrotricha: Chaetonotida). *Marine Biology* 125:735–742. DOI: 10.1007/BF00349256.

Todaro MA., Fleeger JW., Hummon WD. 1995. Marine gastrotrichs from the sand beaches of the northern Gulf of Mexico: species list and distribution. *Hydrobiologia* 310:107–117. DOI: 10.1007/BF00015529.

Todaro MA., Matinato L., Balsamo M., Tongiorgi P. 2003. Faunistics and zoogeographical overview of the Mediterranean and Black Sea marine Gastrotricha. *Biogeographia* 24:131–160.

Todaro MA., Rocha CEF. 2004. Diversity and distribution of marine Gastrotricha along the northern beaches of the state of São Paulo (Brazil), with description of a new species of Macrodasys (Macrodasyida, Macrodasyidae). *Journal of Natural History* 38:1605–1634. DOI: 10.1080/0022293031000156169.

Todaro MA., Rocha CEF. 2005. Further data on marine gastrotrichs from the State of São Paulo and the first records from the State of Rio de Janeiro (Brazil). *Meiofauna Marina* 14:27–31.

Valkanov A. 1957. Erster Versuch zur Erforschung der Gastrotrichen des Schwarzen Meeres. *Annuaire de l'Université de Sofia, Faculté de Biologie, Géologie et Géographie.* Biologie 50:383–399.

Vogt L., Bartolomaeus T., Giribet G. 2010. The linguistic problem of morphology: structure versus homology and the standardization of morphological data. *Cladistics* 26:301–325. DOI: 10.1111/j.1096-0031.2009.00286.x.

Wilke U. 1954. Mediterrane Gastrotrichen. *Zoologische Jahrbücher Abteilung für Systematik Ökologie und Geographie der Tiere* 82:497–550.

International Commission on Zoological Nomenclature, 1999. *International Code of Zoological Nomenclature, Fourth Edition: adopted by the International Union of Biological Sciences*. The International Trust for Zoological Nomenclature

Chapter II – Evolution of the muscular system in the family Xenotrichulidae (Gastrotricha: Chaetonotida) reveals 2 candidate species of *Rotunduxenotrichula intermedia*[2]

Abstract.

The apparent incongruence between widespread distribution of microscopic animals and their life history is known as the ‘‘meiofauna paradox’’. This concept has been investigated in many meiofaunal groups and recent studies provide new insights revealing that the restricted distribution of these organisms is more common than was previously thought based on molecular diversity and muscular organization. In the present study, we investigate the organization of the muscular system in *Rotunduxenotrichula intermedia* from the USA and compare these results to specimens from Kuwait and Italy to determine if muscular organization shows evidence of differences among potentially cryptic species. Four different muscular organizations were identified among populations. Most differences were present in the number and disposition of dorsoventral muscles and the presence of o high numbers of somatic circular muscles. Ours results reinforce the hypothesis that *R. intermedia* represent a cryptic species group made up of at least two candidate species.

Keywords: Muscles; Cryptic species; Gastrotricha; cLSM, cosmopolitan; meiofauna paradox.

[2] Este capítulo está formatado de acordo com as normas fornecidas pela Zoological Journal of the Linnean Society

Introduction

The benthic marine environment houses a great number and diversity of meiofaunal taxa. These microinvertebrates are recognizable by the small size, the short life cycle, lack of dispersal stages, a limited ability to swim, and a discontinuous distribution in the distinct habitats (Higgins & Thiel, 1988; Boeckner, Sharma, & Proctor, 2009; Giere, 2009). These features lead us to believe that the distribution patterns of meiofaunal species would be restricted. However, for many years the existence of biogeographic barriers to meiofaunal dispersal have been examined, and the question remains why many meiofaunal taxa appear to have a near global distribution. This conundrum is called the "meiofauna paradox" (Finlay & Fenchel, 2004; Giere, 2009). Recent surveys using new techniques of morphological and molecular analysis reveal that for many meiofaunal taxa (Leasi & Todaro, 2009; Fontaneto & Hortal, 2012; Kieneke, Martínez Arbizu, & Fontaneto, 2012; Leasi, Andrade, & Norenburg, 2016) the 'paradox' is linked with taxonomic misidentifications due the lack of knowledge of the taxon, instead of a worldwide distributed taxa. The apparent absence of clear morphological delimitations results in our awareness of cryptic species complexes, although further explorations of these complexes based based on genetic, ecological or other morphological (non-traditionally taxonomic) data may actually reveal differences that were previously overlooked (de León & Nadler, 2010).

Among meiofauna groups, the phylum Gastrotricha is commonly found in marine and freshwater habitats all around the world (Balsamo & Todaro, 2002; Balsamo *et al.*, 2008; Balsamo, Guidi, & D'Hondt, 2013; Balsamo *et al.*, 2015; Garraffoni *et al.*, 2016a; Kolicka *et al.*, 2016; Kolicka, 2017). The taxon is classically divided into two orders, Macrodasyida and Chaetonotida (but see Kieneke et al. 2008). The order Chaetonotida is characterized by tenpin-shaped body species spread along 30 genera in eight families (Balsamo *et al.*, 2009, 2015; Hummon & Todaro, 2010; Garraffoni *et al.*, 2016a). This order shows two-thirds of it species living in freshwaters habitats (Balsamo & Todaro, 2002; Balsamo *et al.*, 2013, 2015; Garraffoni *et al.*, 2016a).

In the last years, different methods have been used to study the "meiofauna paradox" in gastrotrichs. Kieneke et al. (2012) used DNA taxonomy (mitochondrial cytochrome c oxidase subunit I gene) to assess the level of cryptic diversity among species belonging to the genus *Turbanella* and showed the occurrence of widely distributed haplotypes related to long-distance dispersal. Garraffoni et al. (2016b) used an ecological approach when they analyzed the variability of marine Gastrotricha diversity along the Brazilian coast and revealed that the diversity patterns of these animals were explained by sediment textures, tidal zones, and localities. Todaro et al (1996) and Leasi and Todaro (2009) analyzed DNA taxonomy/restriction fragment length polymorphisms and muscular systems, respectively, and revealed that *Xenotrichula intermedia* Remane, 1934 (now *Rotunduxenotrichula intermedia*, see Chapter 1) is possibly a cryptic species. These studies provide a large number of data that desmonstrate the strong presence of “meiofauna paradox”on gastrotrichs.

In this present study, we continue to investigate the case of cryptic species in *Rotunduxenotrichula intermedia* by analyzing the muscular architecture of new populations of *R. intermedia* from the USA. We provide a comparison of morphological variation in the muscular system in our findings with other species of Xenotrichulidae. Thus, we were able to establish the morphological variation between *R. intermedia* populations and on the phylogenetic lineages of Xenotrichulidae.

Material and Methods

Sampling

Specimens were sampled from sandy sediments from the sublitoral from the different beachs of Hampton, New Hampshire: South Hampton Beach - SHB (42°53'43"N - 70°49'13"W), same sampling site of Hochberg and Litvaitis (2003), and North Hampton Beach - NHB (42°55'58.84"N – 70°47'48.65"W). The sites are about 8 km distant and possess a different physiology, South Hampton Beach is a common sand beach and North Hampton beach is mix of rock and sand beach. Sediments were placed in plastic bags and brought back to the University of Massachusetts Lowell for analysis.

We collected the top 10 cm of sediment by hand.Meiofauna was extracted from the sediment with isotonic $MgCl_2$ using the anesthetization-decantation technique (Pfannkuche & Thiel, 1988) in 1 L plastic Erlenmeyer flasks. Animals were decanted onto 35 µm sieves and washed with ambient seawater into Petri dishes. Meiofauna were observed alive with Leica EZ4 stereomicroscopes and then transferred to glass slides for specific identification on a Zeiss A1 compound microscope equipped with DIC and Sony Handycam digital video cameras. All gastrotrichs were measured with an ocular micrometer. The position of anatomical characters is provided in percent units (U) of total body length (anterior tip of body (excluding cilia) = U00, posterior tip of body = U100).

Confocal microscopy

Specimens were fixed in 4% paraformaldehyde in 0.1 M saline phosphate buffer (pH 7.2) for at least one week. Specimens were then rinsed in PBS and stained in Alexa Fluor 488 phalloidin (Life Technologies) to document the musculature. Stained specimens were briefly rinsed in PBS before mouting in Fluoromount G (Southern Biotechnology Associates, Birmingham, AL) on glass slides. An Olympus FV 300 confocal laser-scanning microscope was used to visualize the specimens. Ar argon laser (488 nm) was used to excite the samples, and Olympus software was used to capture the images. Confocal z-stacks were collected and processed in as .TIF files and .MOV video files. Files were further processed with Volocity (Perkin Elmer) to generate z-projections.

Scanning electron microscopy

For scanning electronic microscopy (SEM) analysis, two technique were used::

Critical Point Drying: specimens fixed in 2.5% glutaraladehyde in 0.1M sodium cacodylate buffer (pH 7.4) for several days prior to processing. Specimens were then rinsed in 0.1 M buffer for 1h, postfixed in 1% OsO_4 in 0.1M buffer for 1 hr, and then rinsed again in buffer prior to a standard ethanol dehydration series. Specimens were next critical-point dried (Tousimis Samdri-795) and coated with gold using a sputter coater (Denton Vacuum Desk IV) prior to examination with an Amray 1400 SEM at the University of Massachusetts Lowell.

Specimens analyzed

Based on our results in Chapter 1, the family Xenotrichulidae is now divided into four genera, *Draculiciteria*, *Xenotrichula*, *Minutuxenotrichula* and *Rotunduxenotrichula*. To evaluate and compare the muscular system of xenotrichulids we used data from Leasi and Todaro (2008, 2009) for *R. intermedia* populations from Kuwait and Italy, Leasi and Todaro (2008) for *R.*

punctata and *X. squamosa* and Hochberg and Litvaitis (2001) for *Draculiciteria tesselata*. *R. intermedia* specimens from USA were measured alive.

Morphometric analysis

An exploratory principal component analysis (PCA) was carried out on Past software version 2.17 (Hammer, Harper, & Ryan, 2001) to compare measurements obtained by all specimens founded in NHB and SHB (Table 1).

Results

Measurement comparison

Species from SHB present larger specimens (except SHB1 and SHB4) than NHB. The analysis provides that NHB specimens are more closely aligned with each other than SHB specimens. However, principal component analysis could not distinguish specimens from SHB and NHB (Fig. 1).

Table 1 - Measures of *Rotunduxenotrichula intermedia* specimens from North Hampton beach (NHB) and South Hampton Beach (SHB).

	NHB 1	NHB 2	NHB 3	NHB 4	NHB 5	NHB 6	SHB 1	SHB 2	SHB 3	SHB 4	SHB 5	SHB 6	SHB 7
Total body length	175	190	165	125	187.5	175	130	287.5	237.5	111	245	275	237.5
Pharynx length	40	47.5	40	37	42.5	42.5	42.3	67.8	48.8	24.8	56.9	61.5	58.6
Furca length	35.5	35.5	27.5	29.9	35	32.5	33	40	34.2	14.7	36.9	41	34.4
Adhesive tube length	15	15	15	16.5	17.5	15	20	23	17.58	8.6	22.5	22.5	20.9
Pharynx anterior diameter	8	9.8	7	6.3	6.9	8	6	11	6.9	3.9	9.2	8	9.4
Pharynx middle diameter	6	7	6.1	4.2	4.7	6	4.5	9.2	6	3.5	7.1	6	7.7
Pharynx posterior diameter	8.5	10.9	8.9	6.5	8	7.6	7.5	14.4	10	3.7	8.7	13.6	9.8
Head width	29.3	32.8	28	26.7	28.3	32.7	28.8	42.3	35.9	14.6	34.7	40	36.9
Neck width	20	23	18	17	16.9	21.3	16.5	34.9	24.2	10.7	22.4	29.6	25.3
Trunk width	44.6	47	36	23.5	36.6	33.8	20.7	57.8	46.7	16.4	55	63.4	48
Base furca width	17	23	17	13	18	19.4	14.4	30.5	24.3	12.04	28.3	27.2	25
Anus distance from indentation between furcal branches	21	22.9	19.5	13.9	22.8	23	15.3	29.4	30.8	10.5	30.8	36.3	28
Mouth diameter	4.4	4.8	4.9	4.8	3.9	4.8	5	5.3	4	3	4	5	4
No. of dorsal columns of scales	17	18	20	16	19	19	18	20	17	17	18	20	19
No. of scales in dorsal median column	48	53	49	46	54	50	51	51	49	48	50	49	51

No. of scales covering inner margin furca	5	5	5	5	5	5	5	5	5	5	5	5	5

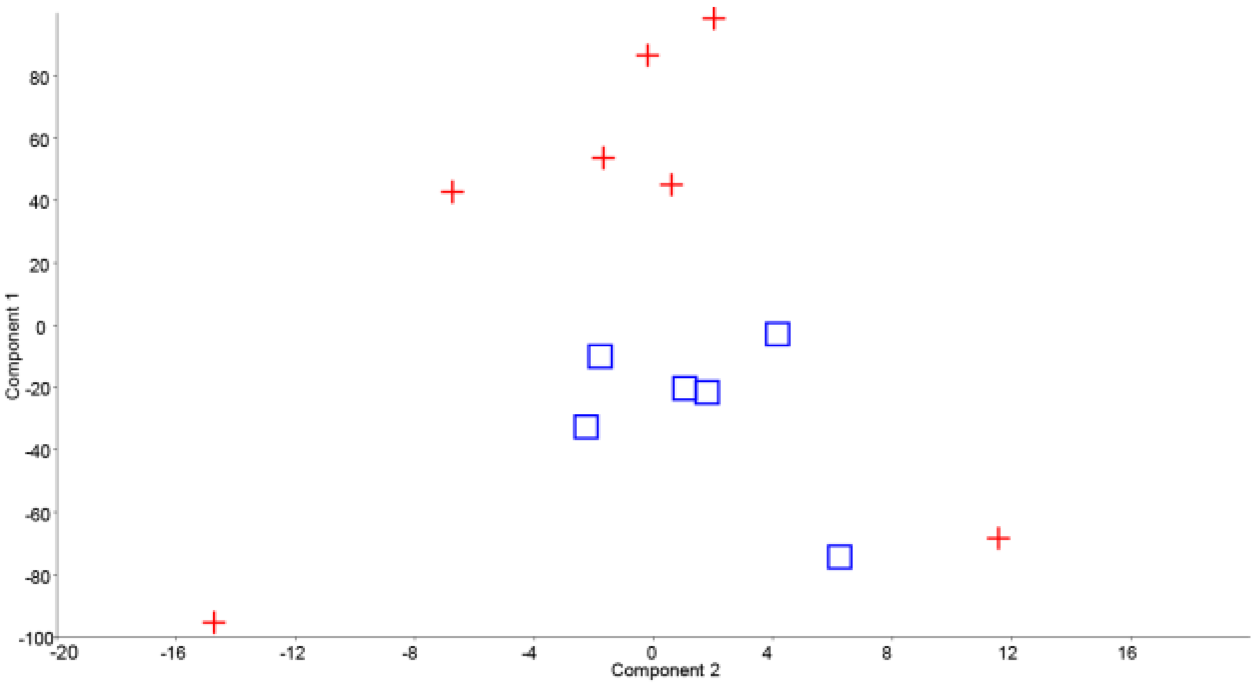

Figure 1 - Graphic of principal component analysis. Blue squares are NHB specimens and red cross are SHB specimens

Muscular system of *Rotunduxenotrichula intermedia* of SHB population

Hochberg and Litvaitis (2003) described the muscular system of *Rotunduxenotrichula intermedia* of the same sampling site of SHB population. The authors used conventional epifluorescence microscopy, which possesses visual limitations. In our survey, confocal microscopy revealed more details: 1) that all circular muscles in the splanchnic position along intestine length are incomplete; 2) the presence several incomplete somatic muscles along the trunk, and 3) the insertion positions of longitudinal muscles can be described with accuracy.

Circular muscles: Present in splanchnic and somatic positions. In splanchnic position, the circular muscles (cm) are complete and surround the entire pharynx from the mouth to the pharyngeointestinal junction (Figs. 2B-C). Along the intestine, the circular muscles are incomplete (icm) and open onto the dorsal and ventral side with a breach on the each end (Figs 2B-D). On the somatic position, the semicircular muscles are present from the pharyngeointestinal junction to the base of the furca (Fig. 2B). There are approximately 50 muscle fibres and surround all the longitudinal muscles except the lateral and ventrolateral muscles longitudinal muscles.

Longitudinal muscles: Present in splanchnic and somatic position. In the splanchnic position, two pairs of longitudinal muscles are in the ventral portion of the body (Fig. 2C). One pair is present in a ventral position (vm) and extends from the furca base to the pharynx; these muscles cross each other close to the furca base. Another pair of longitudinal muscles are present in the ventromedial (vmm) region; these muscles arise from the lateral furca base, divide close to the anus and extend parallel until to the anterior portion of the intestine where they join again and insert on the mouth rim. On the dorsal portion, a pair of longitudinal muscles (dm) is inserted on the mouth rim and extends to the furca base (Fig. 2B).

In the somatic position, two pairs of longitudinal muscles are present. One pair of lateral muscles (lm) extended from the dorsal mouth rim and inserted laterally to the furca muscles. One pair is present in the ventrolateral position (vlm), extending from the lateral of furca to pharynx, where they split at half furca, one branch inserting on the mouth rim and the other extending parallel to the pharynx inserting in the anterior cuticle (Figs. 2B-C).

Helicoidal muscles: Two pairs of muscles in a helicoidal arrangement (hm) extend from ca. first third of the pharynx to the anterior portion of the intestine (Figs. 2B-D).

Dorsoventral muscles: The dorsalventral muscles (dvm) are present in between the intestine and lateral body wall from the approximately the anterior third of the intestien to the furca base. The muscles tips are branched and inserted in the cuticle. The most lateral dorsoventral muscles are curved and the others on the lateral of intestine are straight (Figs. 2B, C, E).

Furcal muscle arrangements: All the furca branches are wrapped by complete circular muscles and three pairs of dorsoventral muscles.

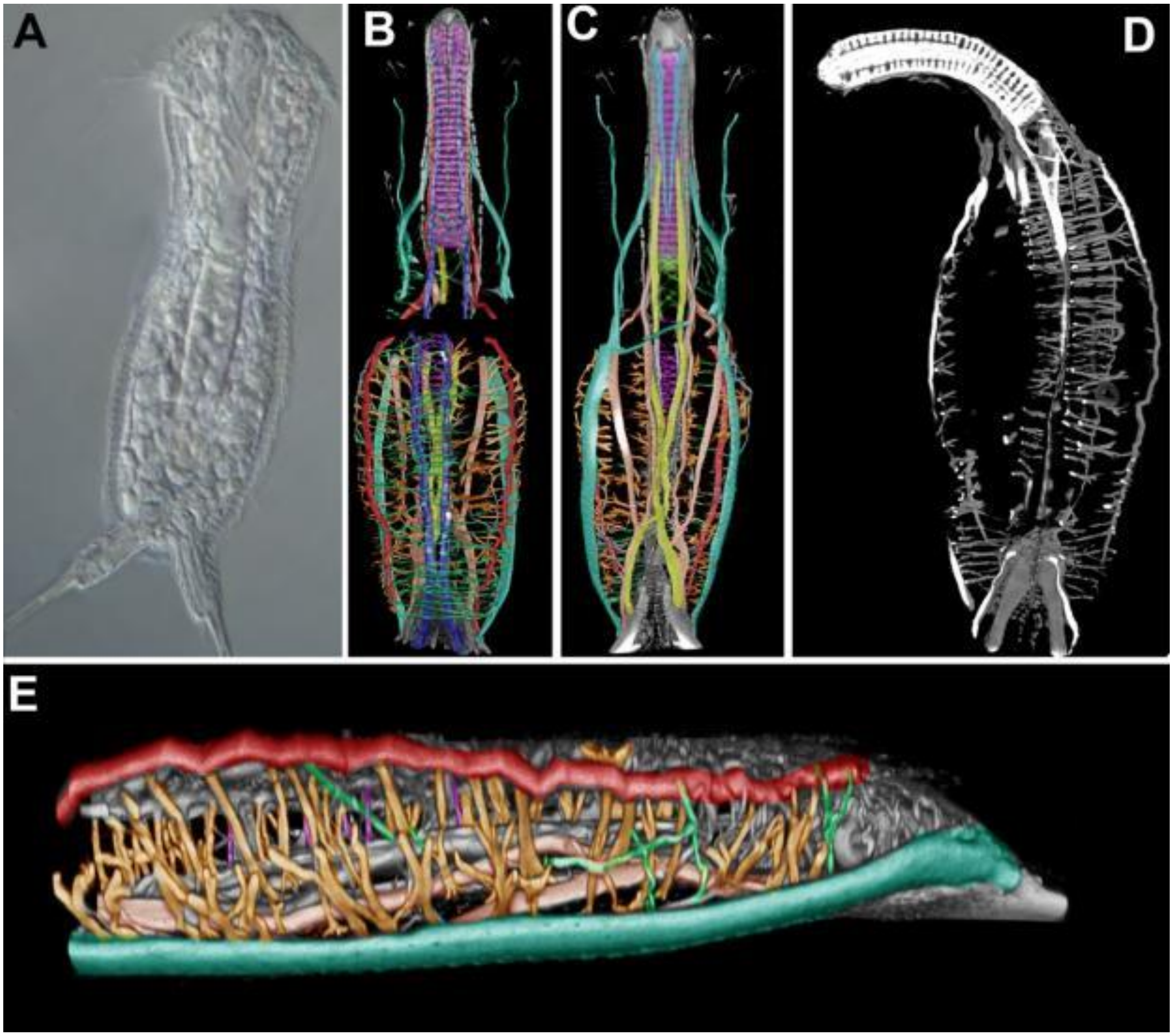

Figure 2 - A, Ventral habitus of *R. intermedia* South Hampton beach. B – E: Confocal photomicrograph of a phalloidin-stained specimen showing the muscular system. B, Volocity-rendered view of muscular organization of dorsal side. C, Volocity-rendered view of muscular organization of ventral side. D, Volocity-rendered view of incomplete circular muscles of splanchnic portion. E, Volocity-rendered view of muscular organization in lateral view. Colors: Pink, circular muscles of splanchnic portion; Green, circular muscles of somatic portion; Orange, dorsoventral muscles; Bright green, helicoidal muscles; Light orange, ventral longitudinal muscles; Yellow, ventromedial longitudinal muscles; Blue, dorsal longitudinal muscles; Red, lateral longitudinal muscles; Light green, ventrolateral longitudinal muscle.

Muscular system of *Rotunduxenotrichula intermedia* – NHB population

Circular muscles: Present in splanchnic and somatic positions. In splanchnic position the circular muscles (cm) are complete and surround the entire pharynx from the mouth to the pharyngeointestinal junction (Figs 3B-C). Along the first half of the intestine, the circular muscles are incomplete (icm) and open onto the dorsal and ventral side with a breach on each end (Figs 3B-C). On the somatic position the two or three semicircular muscles are present on the second half of intestine (Fig. 3B - C) and surround all the other muscles (Fig. 3D).

Longitudinal muscles: Present in splanchnic and somatic positions. In the splanchnic position, two pairs of longitudinal muscles are present in the ventral portion (Fig. 3C). One pair is present in a ventral position (vm) and extends from the furca base to the pharynx; close to the furca these muscles cross each other and extend until mouth rim. One pair of longitudinal muscles is present in a ventromedial (vmm) position; these muscles arise on the lateral furca base and extend parallel until the mouth rim. On the dorsal size, a pair of dorsal longitudinal muscles (dm) is inserted on the mouth rim and extends to the furca base (Fig. 3C).

In the somatic position, two pairs of longitudinal muscles are present. One pair of lateral muscles (lm) extended from the dorsal mouth rim and inserted laterally to the furca muscles. These muscles possess a small branch at pharyngeointestinal junction and involve the dm. One pair is present in the ventrolateral position (vlm), extending from the ventral portion of furca to pharynx, where they split at half furca, one branch inserting on the mouth rim and the other run parallel to the pharynx inserting in the anterior cuticle (Figs. 3B-C).

Helicoidal muscles: Two pairs of muscles in a helicoidal arrangement (hm) extend from the anterior third of the pharynx to the anterior portion of the intestine (Figs. 3B-C).

Dorsoventral muscles: The dorsoventral muscles (dvm) are present lateral to the intestine, from the posterior third of the intestine to the furca base and some pairs on the pharyngeointestinal junction. The muscles tips are branched and inserted in the cuticle. They are arranged in six pairs in the intestine region and four pairs on the pharyngeointestinal junction region (Figs. 3B-D)

Furcal muscle arrangements: All the furca branches are wrapped by complete circular muscles and three pairs of dorsoventral muscles.

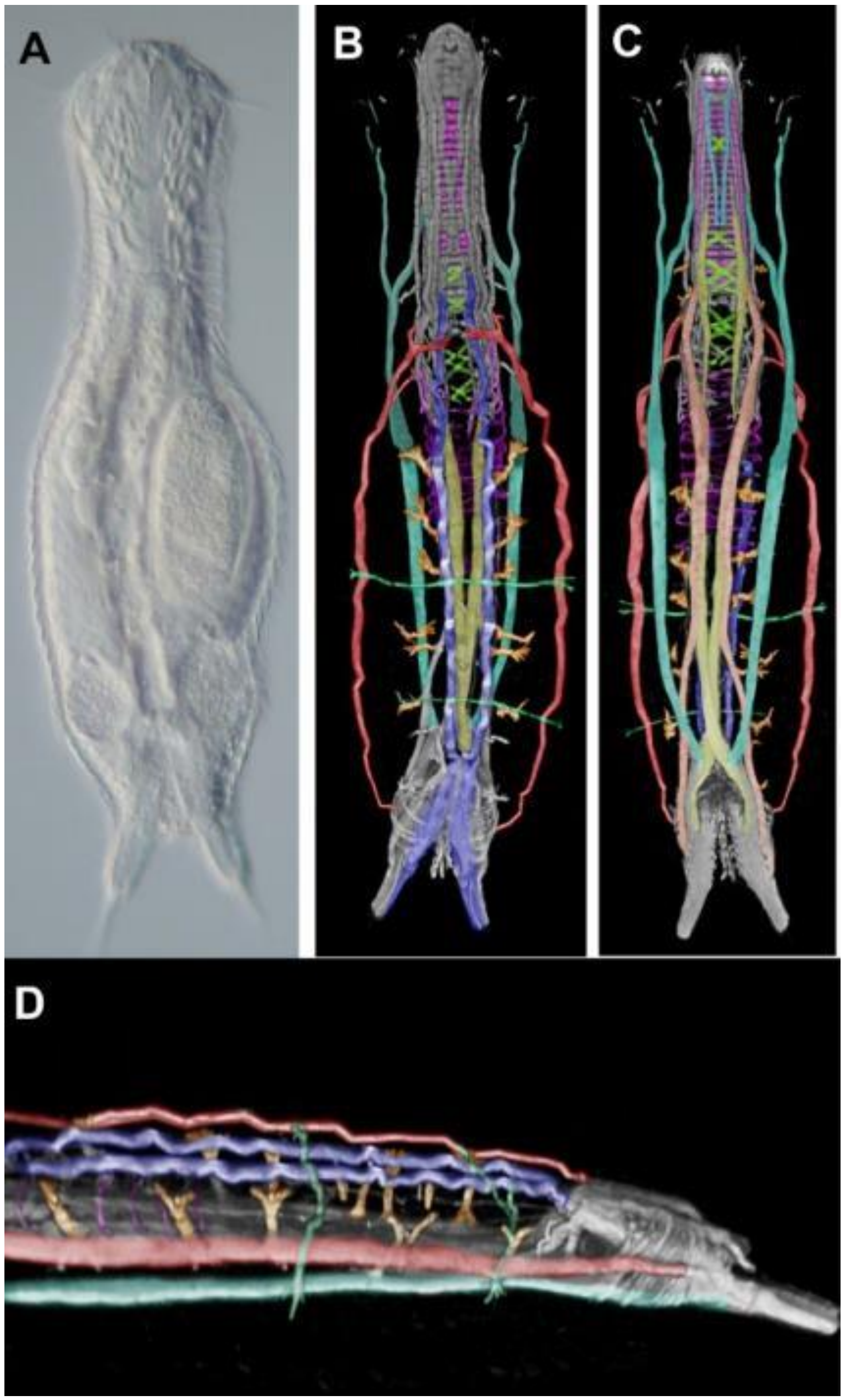

Figure 3 - A, Ventral habitus of *R. intermedia* North Hampton beach. B – D: Confocal photomicrograph of a phalloidin-stained specimen showing the muscular system. B, Volocity-rendered view of muscular organization of dorsal side. C, Volocity-rendered view of muscular organization of ventral side. D, Volocity-rendered view of muscular organization in lateral view. Colors: Pink, circular muscles of splanchnic portion; Green, circular muscles of somatic portion; Orange, dorsoventral muscles; Bright green, helicoidally muscles; Light orange, ventral longitudinal muscles; Yellow, ventromedial longitudinal muscles; Blue, dorsal longitudinal muscles; Red, lateral longitudinal muscles; Light green, ventrolateral longitudinal muscle.

Muscular system of *Rotunduxenotrichula intermedia* from Kuwait and Italy populations, *R. puntacta*, *Xenotrichula squamosa* and *Draculiciteria tesselata*

The muscular system of *Rotunduxenotrichula intermedia* populations from Italy (Fig. 5G-H) and Kuwait (Fig. 5E-F) were obtained from Leasi and Todaro (2008, 2009); *R. puntacta* (Fig. 4A) and *Xenotrichula squamosa* (Fig. 4B) were extracted from Leasi and Todaro (2009); *Draculiciteria tesselata* was extracted from Hochberg and Litvaitis (2001) and from personal observations (Fig. 5C).

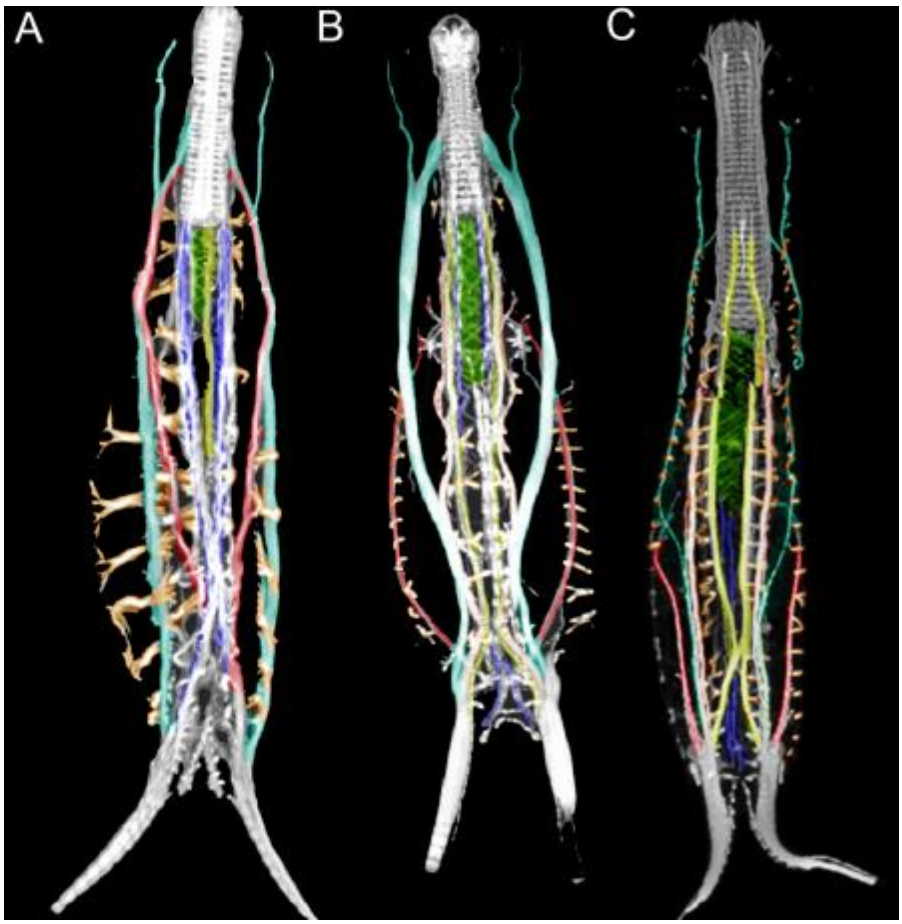

Figure 4 – Confocal photomicrograph of a phalloidin-stained specimen showing the muscular system. A, *Rotundunxenotrichula punctata*. B, *Xenotrichula squamosa.* C. *Draculiciteria tesselata.* A-B: Modified from Leasi and Todaro (2008). Colors: Pink, circular muscles of splanchnic portion; Green, circular muscles of somatic portion; Orange, dorsoventral muscles; Bright green, helicoidally muscles; Light orange, ventral longitudinal muscles; Yellow, ventromedial longitudinal muscles; Blue, dorsal longitudinal muscles; Red, lateral longitudinal muscles; Light green, ventrolateral longitudinal muscle. A-B: Modified from Leasi and Todaro (2008)

Overall comparison of *Rotunduxenotrichula intermedia* muscular system between USA populations

The muscular architecture of USA populations from NHB and SHB show several similarities: specimens from both populations present large splanchnic dorsoventral muscles; similar insertions of ventromedial longitudinal muscles; the heliocoidal muscles extending to U48; the presence of incomplete somatic muscle and the splanchnic circular muscles along the intestine length are incomplete in both populations.

On the other hand, the differences between specimens from SHB and NHB are obvious. The incomplete somatic circular muscles in the SHB populations are present in higher number than the NHB population and they do not surround all the longitudinal muscles. The ventromedial longitudinal muscles in SHB present a split on the intestine region but in NHB this muscles are straight in all the extension. The insertion of the ventrolateral muscles on the furca base is different in these two populations, in SHB the insertion is on lateral portion of the furca but in the NHB it is on the ventral portion of furca base

SHB specimens resemble those specimens analysed by Hochberg & Litvaitis (2003) due to the presence of dorsoventral muscles in the lateral body region and somatic semicircular muscles on the anterior portion of the intestine. We add to their description the presence of dorsoventral muscles in the lateral region of the intestine and semicircular somatic muscles distributed along the entire trunk region.

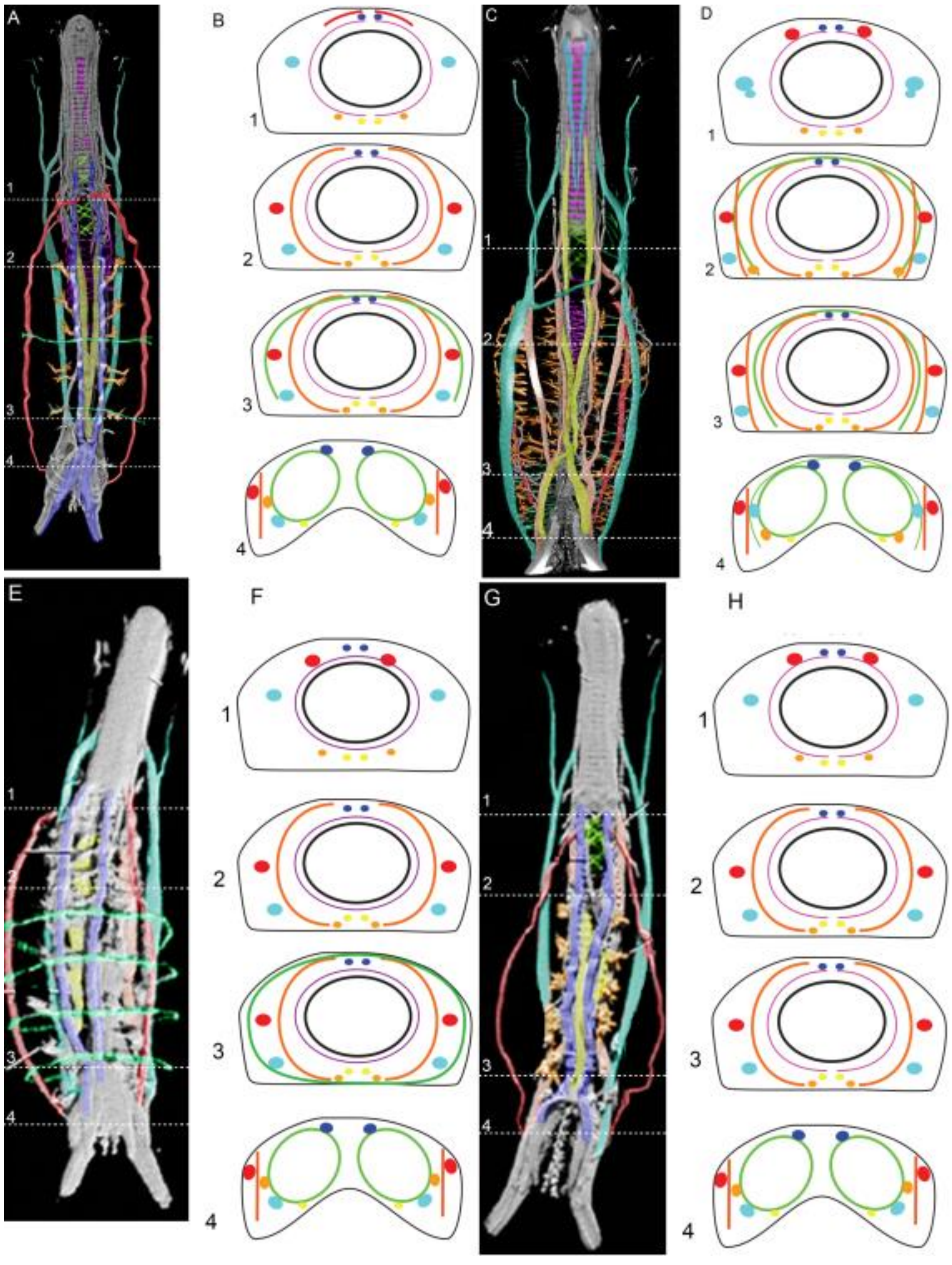

Figure 5 - Schematich draw of tranversal cut along body lenght. A - B, *R. intermedia* NHB specimen. C-D, *R. intermedia* SHB specimen. E-F. *R. intermedia* Kuwait specimen. G-H. *R. intermedia* Italy specimen. Colors: Pink, circular muscles of splanchnic portion; Green, circular muscles of somatic portion; Orange, dorsoventral muscles; Bright green, helicoidally muscles; Light orange, ventral longitudinal muscles; Yellow, ventromedial longitudinal muscles; Blue, dorsal longitudinal muscles; Red, lateral longitudinal muscles; Light green, ventrolateral longitudinal muscle. E-H: Modified from Leasi and Todaro (2009).

Overall comparison of *Rotunduxenotrichula intermedia* muscular system among USA, Italy, and Kuwait populations

The muscular architecture of *R. intermedia* around the world presents several similarities including dorsoventral and semicircular muscles in a splanchnic position, longitudinal muscles of the same size, and ventromedial muscles inserted on the ventral furca. On the other hand, the present study reveals four different patterns of muscular architecture among specimens of *R. intermedia.*

The first two patters are observed in NHB and Italy populations. These populations present similar organizations with the longitudinal muscles and dorsoventral muscles present in identical positions. The differences between these two populations are in the presence of incomplete somatic circular muscles in NHB specimens and the anterior portion of the lateral longitudinal muscles on the NHB population become a branch instead a continuous tube as observed in Italian populations.

The third organization is observed in Kuwait specimens. The muscular structure resembles NHB and Italian architectures but diverges in the presence of complete somatic and splanchnic circular muscles instead incomplete circular muscles in NHB (splanchnic and somatic) and Italian (splanchnic) populations.

The fourth and most different architecture was observed in the SHB population. This specimen presents a higher number of somatic semicircular muscles and dorsoventral muscles on the somatic portion are only observed in this species. The differences between the longitinal muscles of SHB and others populations are on the insertion in the furca: ventral longitudinal and ventrolateral longitudinal muscle insert on the lateral furca. In this population each ventral longitudinal muscles split in two branches at anus portion and join at the first third of intestine region.

Overall comparisons between Xenotrichulidae species

All xenotrichulids possess a pharynx that is completely surrounded by circular muscles, with dorsoventral, lateral, ventrolateral, ventromedial, ventral and dorsal longitudinal muscles along the splanchnic regions (Fig. 6).

In the family Xenotrichulidae we observe three patterns of circular muscles: exclusively present along the splanchnic region in *X. squamosa* and *R.punctata;* exclusively present along the pharynx in *Draculiciteria tesselata;* and present along the splanchnic portion and somatic portion in *R. intermedia*. The lateral longitudinal muscles present two different arrangements and two different diameters: tubular with anterior branch on the PhInJ region in NHB population and tubular shape in total length observed in all remaining species. *Draculiciteria tesselata* and *Xenotrichula squamosa* have thinner diameter muscules than other xenotrichulids.

All specimens present dorsoventral muscles on the splanchnic and somatic portions, except specimens of *R. intermedia* from NHB (USA), Kuwait and Italy, which present only dorsoventral muscles on the splanchnic portion. *Draculiciteria tesselata* and *Xenotrichula squamosa* have a thinner diameter muscle than other species of Xenotrichulidae. The position of the somatic dorsoventral muscles is usually external of all longitudinal muscles, with the unique exception in the SHB specimens where the ventrolateral and lateral muscles are external of the dorsoventral muscle.

Discussion

In the past several decades, technological advances in microscopy have allowed for a more complete picture of the gastrotrich muscle system, which has in turn gained more notoriety in evolutionary studies of gastrotrichs. The first muscle studies used TEM and with the passage of time came the ability to visualize entire muscular systems in situ (and not reliant on sectional views). These studies used fluorescent markers to label muscles that could then be visualized with widefield epifluorescence microscopy and later confocal laser scanning microscopy, which provided greater resolution and clarity. As pointed out by Leasi and Todaro (2008), epifluorescence has visual limitations that may end up generating errors of interpretation. The cLSM in turn allows the muscles to be viewed in 3D and smaller details to be accessed.

Homologies in the muscular system

On the muscular system of Xenotrichulidae species, the circular and dorsoventral muscles present the largest variation in the family. The diversification of these components can be linked to the natural history of these animals.

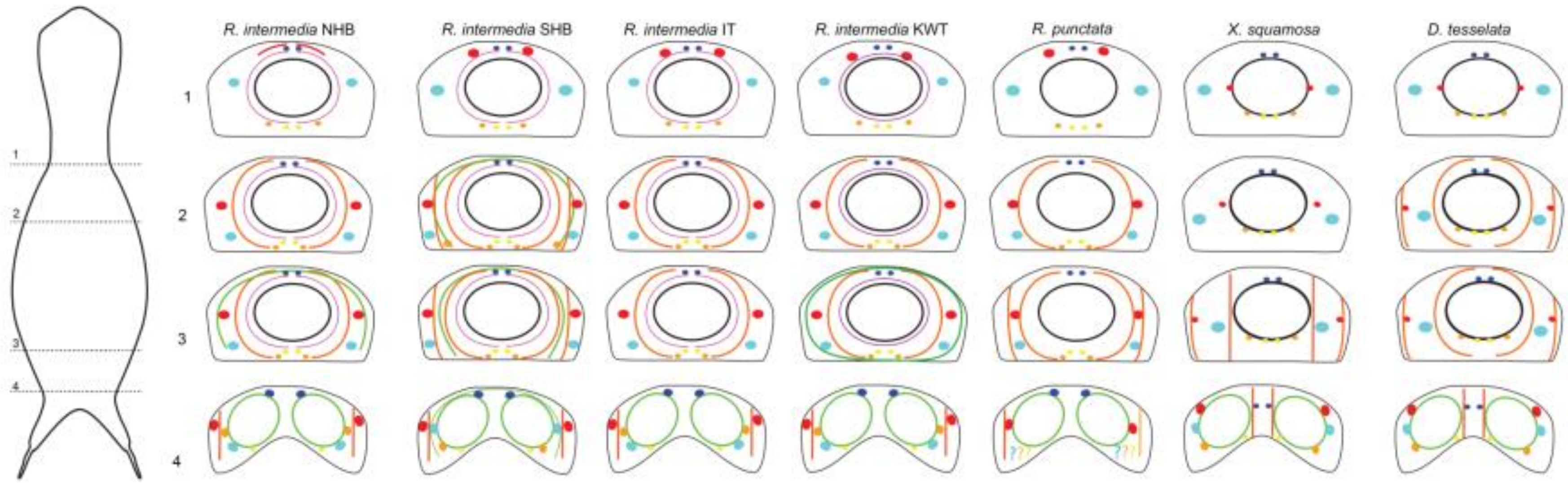

Figure 4 - Schematic draw of transversal cuts on Xenotrichulidae species. Colors: Pink, circular muscles of splanchnic portion; Green, circular muscles of somatic portion; Orange, dorsoventral muscles; Light orange, ventral longitudinal muscles; Yellow, ventromedial longitudinal muscles; Blue, dorsal longitudinal muscles; Red, lateral longitudinal muscles; Light green, ventrolateral longitudinal muscle.

The presence of dorsoventral muscles function in compression of body in the dorsoventral plane, which may allow for greater mobility between sand grains and in same cases adjustments of egg posture as hypothesized by Hochberg & Litvaitis (2003). According Leasi and Todaro (2008), the dorsoventral muscles are derivatives of the dorsal and ventral openings of complete circular muscles

Our surveys demonstrate a different interpretation for these muscles. The presence of semicircular splanchnic muscles would be evidence of an intermediate stage of the transformation series of dorsoventral muscles. On the other hand, we observe the presence of at least dorsoventral and semicircular muscles in a splanchnic position on *R. intermedia* specimens. Additionally, *R. intermedia* specimens from Kuwait present complete circular muscles in somatic and splanchnic positions and the dorsoventral muscles are present as well. Therefore, it would appear that the circular and dorsoventral muscles evolved independently along the family Xenotrichulidae. We conclude that dorsoventral muscles are not derivatives of circular muscles or circular muscles branches.

Evolutionary view of the muscular system in Xenotrichulidae

Chapter I of this thesis provides a new classification based on the phylogenetic analysis. Using the topology provided by this work, we investigated the evolution of the muscular system in the family (Fig. 7).

The first divergence on the family Xenotrichulidae is between Draculiciterinae and (Xenotrichulinae + Rotundunxenotrichulinae). The muscular architecture of *Draculiciteria* species appears to be a plesiomorphic condition of Xenotrichulidae. The dosoventral muscles (somatic and splanchnic portion) and the lateral muscles are thin in diameter. This arrangement can be observed in the Xenotrichulinae species. *Xenotrichula squamosa* presents the same architecture as *D. tesselata* with just one difference in the splanchnic dorsoventral muscles: *D. tesselata* presents the muscles as curved on the intestine instead of straight as in *X. squamosa.*

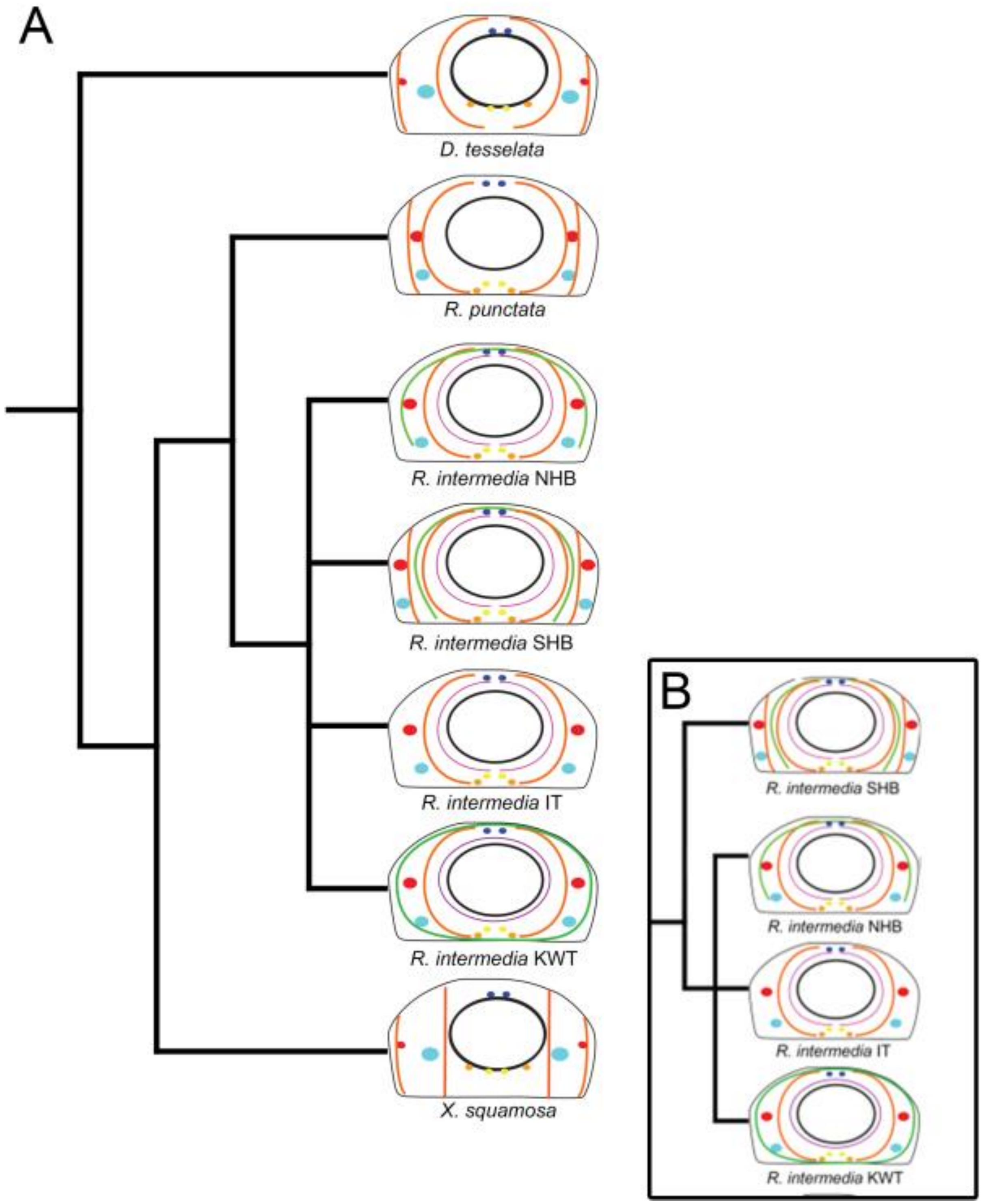

Figure 7 - A - Muscular system of along Xenotrichulidae species. Cladrogram modified from Chapter I. B - Schematic representation of the hypothetical muscular evolutionary scenario on *R. intermedia* complex. Colors means: Pink, circular muscles of splanchnic portion; Green, circular muscles of somatic portion; Orange, dorsoventral muscles; Bright green, helicoidal muscles; Light orange, ventral longitudinal muscles; Yellow, ventromedial longitudinal muscles; Blue, dorsal longitudinal muscles; Red, lateral longitudinal muscles; Light green, ventrolateral longitudinal muscle.

The derived condition of the muscular system can be observed in Rotundunxenotrichulinae. All specimens of this taxon present dorsoventral and lateral muscles with an incremental increase in the diameter. The presences of these muscles on somatic and splanchnic portion are the plesiomorphic condition of this subfamily.

***Rotunduxenotrichula intermedia* is in fact a cryptic species complex**

Analysing the *R. intermedia* complex (Figs. 5-6, 7B) can be observed the presence of the splanchnic circular muscles in all specimens. Specimens from NHB, Italy and Kuwait appear to be the same species presenting only splanchnic dorsoventral muscles and a slight variation on the somatic circular muscles can be observed along these specimens. NHB specimens possess few somatic complete circular muscles wrapping all other muscular components; NHB specimens possess few somatic incomplete circular muscles wrapping all other muscular components and Italian species do not present these components.

Among *R. intermedia* populations, specimens from SHB are unique and present dorsoventral muscles on the somatic and splanchnic portions, but these muscles are internal to the lateral and ventrolateral longitudinal muscles. The somatic circular muscles are internal to lateral and ventrolateral longitudinal and somatic dorsoventral muscles and are present in a higher number along the trunk. In summary, based on the architecture observed in these specimens, we hypothesize that is a different morphotype of *R. intermedia*.

These morphological differences lead us to hypothesize that *R. intermedia* complex includes at least two different morphotypes. The first morphotypes is the species with a large number of semicircular muscles along the entire dorsal trunk portion and the second morphotype possesses few semicircular muscles on the trunk portion, which surround all the others muscles. As reported before, some studies found a high genetic divergence between different *R. intermedia* populations, which reinforces the conclusion that a cryptic *R. intermedia* is in fact a complex of genetic and morphologically different species. A molecular analysis can provide more data and reinforce our hypothesis further. As pointed on the introductory section of this thesis, we are working on the improvement of the protocols to perform it.

Acknowledgments

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References

Balsamo M, D'Hondt JL, Kisielewski J, Pierboni L. 2008. Global diversity of gastrotrichs (Gastrotricha) in fresh waters. *Hydrobiologia* 595: 85–91.

Balsamo M, D`Hondt JL, Kisielewski J, Todaro M, Tongiorgi P, Guidi L, Grilli P, de Jong Y. 2015. Fauna Europaea: Gastrotricha. *Biodiversity Data Journal* 3: e5800.

Balsamo M, D`Hondt JL, Pierboni L, Grilli P. 2009. Taxonomic and nomenclatural notes on freshwater Chaetonotida. *Zootaxa* 2158: 1–19.

Balsamo M, Guidi L, D'Hondt JL. 2013. Phylum Gastrotricha. *Zootaxa* 3703: 79–82.

Balsamo M, Todaro MA. 2002. Gastrotricha. In: Rundle SD,, In: Robertson AL,, In: Schmid-Araya JM, eds. *Freshwater Meiofauna: Biology and Ecology*. Leiden: Backhuys Publishers, 45–61.

Boeckner MJ, Sharma J, Proctor HC. 2009. Revisiting the meiofauna paradox: Dispersal and colonization of nematodes and other meiofaunal organisms in low- and high-energy environments. *Hydrobiologia* 624: 91–106.

Finlay BJ, Fenchel T o. m. 2004. Cosmopolitan Metapopulations of Free-Living Microbial Eukaryotes. *Protist* 155: 237–244.

Fontaneto D, Hortal J. 2012. Microbial biogeography: is everything small everywhere? In: Ogilvie LA,, In: Hirschl PR, eds. *Microbial Ecological Theory: Current Perspectives*. Norwich: Horizon Scientific Press, 87–98.

Garraffoni ARS, Araújo TQ, Lourenço AP, Guidi L, Balsamo M. 2016a. A new genus and new species of freshwater Chaetonotidae (Gastrotricha: Chaetonotida) from Brazil with phylogenetic position inferred from nuclear and mitochondrial DNA sequences. *Systematics and Biodiversity*.

Garraffoni ARS, Di Domenico M, Amaral ACZ. 2016b. Patterns of diversity in marine Gastrotricha from Southeastern Brazilian Coast is predicted by sediment textures. *Hydrobiologia* 773: 105–116.

Giere O. 2009. *Meiobenthology*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Hammer Ø, Harper DAT a. T, Ryan PD. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4(1): 1–9.

Higgins RP, Thiel H. 1988. *Introduction to the study of meiofauna* (RP Higgins and H Thiel, Eds.). Washington, D.C.: Smithsonian Institution Press,.

Hochberg R, Litvaitis MK. 2001. The musculature of *Draculiciteria tessalata* (Chaetonotida, Paucitubulatina): implications for the evolution of dorsoventralmuscles in Gastrotricha. *Hydrobiologia* 452: 155–161.

Hochberg R, Litvaitis MK. 2003. Organization of muscles in Chaetonotida Paucitubulatina (Gastrotricha). *Meiofauna Marina* 12: 47–58.

Hummon WD, Todaro MA. 2010. Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. *Zootaxa* 32: 1–32.

Kieneke A, Martínez Arbizu P, Fontaneto D. 2012. Spatially structured populations with a low level of cryptic diversity in European marine Gastrotricha. *Molecular Ecology*: 1239–1254.

Kieneke A, Riemann O, Ahlrichs WH. 2008. Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. *Zoologica Scripta* 37: 429–460.

Kolicka M. 2017. New Arctic Gastrotricha — Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). *Annales Zoologici* 67: 131–180.

Kolicka M, Dabert M, Dabert J, Kånneby T, Kisielewski J. 2016. Bifidochaetus, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. *Invertebrate Systematics* 30: 398.

Leasi F, Andrade SCDS, Norenburg J. 2016. At least some meiofaunal species are not everywhere. Indication of geographic, ecological and geological barriers affecting the dispersion of species of *Ototyphlonemertes* (Nemertea, Hoplonemertea). *Molecular Ecology* 25: 1381–1397.

Leasi F, Todaro MA. 2008. The muscular system of *Musellifer delamarei* (Renaud-Mornant, 1968) and other chaetonotidans with implications for the phylogeny and systematization of the Paucitubulatina (Gastrotricha). *Biological Journal of the Linnean Society* 94: 379–398.

Leasi F, Todaro MA. 2009. Meiofaunal cryptic species revealed by confocal microscopy: the case of Xenotrichula intermedia (Gastrotricha). *Marine Biology* 156: 1335–1346.

de León GPP, Nadler SA. 2010. What we don't recognize can hurt us: A plea for awareness about cryptic species. *Journal of Parasitology* 96: 453–464.

Pfannkuche O, Thiel H. 1988. Sampling processing. *Introduction to the Study Meiofauna*.134–145.

Chapter III - Description of Xenotrichula sp. nov. (Gastrotricha: Chaetonotida: Xenotrichulidae) from Brazilian coast.[3]

Abstract

A new species of *Xenotrichula* (Gastrotricha: Chatonotida: Xenotrichulidae) is sublittoral zones of Brazilian northeast (state of Bahia) and southeast (state of São Paulo) coasts. *Xenotrichula* sp. nov. is unique among its co-generic species in possessing the ventral pharynx length covered by double edge rounded scales and two rows of flat plates on the ventral trunk. This is the first record for the family in the Brazilian northeast region.

Keywords. New species; meiofauna; Gastrotricha; Bahia State; São Paulo State; marine.

[3] Este capítulo está formatado de acordo com as normas fornecidas pela revista Marine Biodiversity.

Introduction

Gastrotricha is a group of free-living microinvertebrates present in freshwater, estuarine and marine environments (Balsamo and Todaro 2002; Balsamo et al. 2009). The taxon includes about 820 species (Araújo et al. 2015; Kieneke et al. 2015; Balsamo et al. 2015; Kolicka et al. 2015, 2016; Garraffoni et al. 2016; Kånneby 2016; Kolicka 2017) grouped into two classic orders, Macrodasyida Remane 1925 [Rao and Clausen 1970] and Chaetonotida Remane 1925 [Rao and Clausen 1970] (but see Kieneke et al. 2008).

Belonging to the order Chaetonotida Gosse, 1864, the marine family Xenotrichulidae was described by Remane (1927) based on the ventral ciliation restricted on the pharynx region, the dorsal body covered by pedunculate scales and the paired testes.

For long time, this family was divided into three genera (Ruppert 1979; Todaro 2017): *Draculiciteria* (Hummon 1974), *Heteroxenotrichula* (Wilke 1954), and *Xenotrichula* (Remane 1927). However, recently, after a phylogenetic reconstruction including all Xenotrichulidae species a new classification at genus level was proposed and two new genera were created (*Minutuxenotrichula* and *Rotunduxenotrichula*), *Heteroxenotrichula* was synonymized with *Xenotrichula*, and only *Draculiciteria* remains unchanged (chapter I of this thesis). In this new arrangement proposed in the chapter 1, *Xenotrichula* is the most speciose genus of the family with 12 species supported by the following characters: lateral tentacles on head, dorsal body covered by subterranea-type scales, and locomotory cirri of different sizes (chapter 1).

During one of the first surveys of gastrotrichs on the Brazilian coastline, Todaro and Rocha (2004, 2005) reported the species *Xenotrichula pygmea* from the state of Rio de Janeiro and *X. squamosa* from state of São Paulo. Here we provided a description of the first *Xenotrichula* species endemic to the Brazilian coast. With this new finding, we also tried to shed light on about the distribution patterns of the Brazilian marine gastrotrichs.

Materials and Methods

Sampling

We collected the top 10 cm of sediment by hand (total of 50 liters of sediments) in the sublittoral region from Pau Fincado Beach (17º54' S' - 39º22'W, city of Nova Viçosa, state of Bahia, Brazilian northeast), between 29 December 2013 to 5 January 2014. Sandy sediments were also collected in the sublittoral region Centro Beach (23°38'S - 45°25'W, city of Caraguatatuba, state of São Paulo, Brazilian southeast) in 6 May 2016 (Fig. 1).

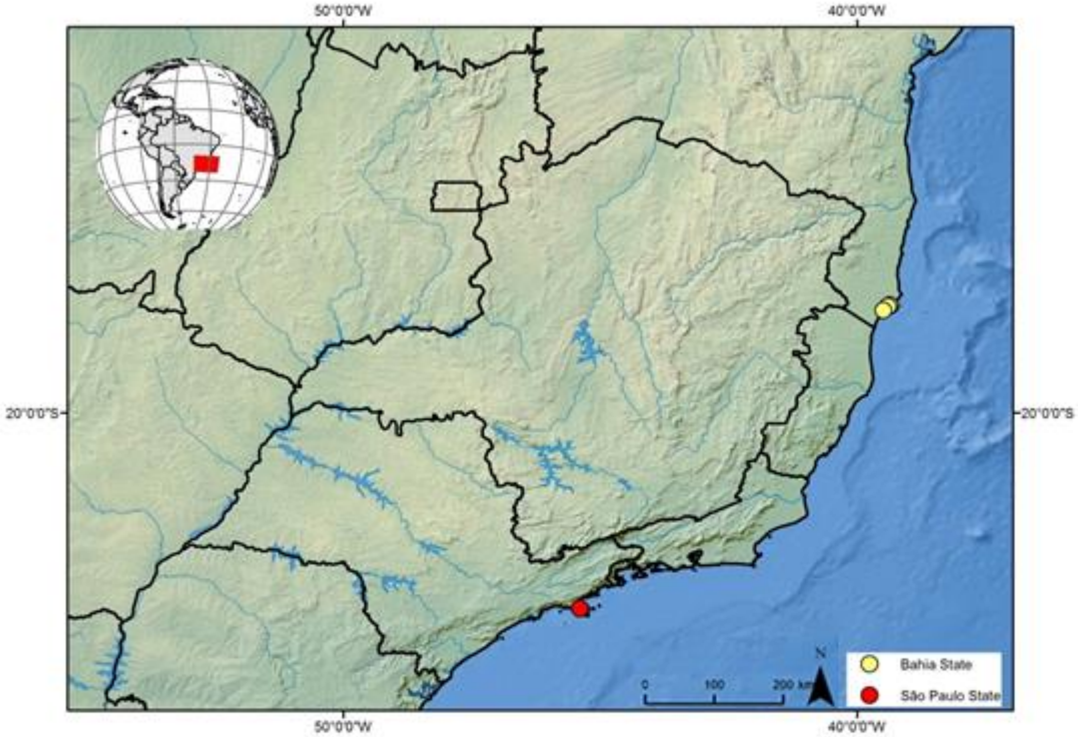

Figure 1 - Schematic map of sampling sites.

Gastrotrichs were located by sorting small amounts of sediment poured into Petri dishes under a Zeiss Stemi 2000 stereomicroscope. The animals were isolated alive and mounted individually on glass slides, anaesthetized with 7% MgCl2 and digitally documented under a Zeiss Axio Imager M2 light microscope equipped with DIC and an AxioCam MRC5 digital video camera. For SEM analysis, some specimens were fixed in 2% glutaraldehyde in cacodylate buffer or 2% paraformaldehyde in PBS, then rinsed in 0.1 M PBS, dehydrated in a graded ethanol series, treated with HMDS (hexamethyldisilazane) (Hochberg and Litvaitis 2000), mounted on aluminum stubs, and sputter-coated with gold-palladium. Observations were carried out under a JSM 5800LV SEM at the University of Campinas (UNICAMP). The positions of morphological characters along the body are given in percentage units (U) of total body length measured from the anterior to the posterior end (Hummon et al. 1992).

Results

Order Chaetonotida Remane, 1925 [Rao and Clausen 1970]
Family Xenotrichulidae Remane, 1927
Subfamily Xenotrichulinae Remane, 1927 [Chapter I]
Genus *Xenotrichula* Remane, 1927 [Chapter I]
Xenotrichula sp. nov.

(figs 2-4)

Diagnosis. A *Xenotrichula* with an adult length to 150 µm; tenpin-shaped body, posterior end furcated. Oval head with anterior mouth, few oral bristles and lateral tufts of sensorial cilia present on the base of tentacles. Lateral long scaled tentacles present. Four pairs of sensory hairs spaced on the lateral side of the body. Cuticular armature of small stalked scales increase size from anterior to posterior end dorsal body. Ventrolateral surface with hydrofoil scales with similar sizes along the body. On the middle of ventral field with 21 transversal plates. Caudal furca with adhesive tubes. Ventral locomotor cilia: two longitudinal columns of continuous locomotory cilia covering the head to pharyngeo-intestinal junction. A pair of tufts are observed in the middle gut region.

Type material. *Holotype*. Adult, collected from sand beach on January 02, 2014,at 0.8 m depth in the Pau Fincado Beach, Nova Viçosa, Brazil (17º54' S - 39º22' W), mounted on glass slide, deposited at the Museu de Zoologia, Universidade Estadual de Campinas, Brazil, under accession number ZUEC GCH 36, *Paratypes* 3 adult specimens, collected at Pau Fincado Beach, mounted on 2 glass slides, deposited at the Museu de Zoologia, Universidade Estadual de Campinas, Brazil, under accession numbers ZUEC GCH 37 and 38.

Description. The description is based on the adult specimen, 150 µm in total length (Figures 2-3). Pharynx 41.5 µm in length; pharyngeo-intestinal junction at U27.6. Head well developed, with an anterior mouth (8 µm of diameter) and two pairs of sensorial tufts. Lateral long scaled tentacles (22.5 µm) present. Neck constriction moderate (21µm), furca at U72. Scaled furca basis longer (34 µm) than adhesive tubes (16µm). Four pairs of sensorial bristles on the dorsal body (9 µm, U22, U34, U51, U62). A pair of sensorial bristles on the base of furca

(U73). Paired transversal rows of cilia extending from each side of mouth to tentacle tip (previously named as *Kammantiger Zacken*, see chapter I).

Cuticular armature: Cephalion very small (3.8 µm) imbricated on the epidermis; two hard projection (previously named as hypostomiun, see chapter I) on each side mouth present (1.5µm); 14 rows of pedunculated, somewhat large triangular scales with rounded edges supported by a pedunculum inserted on a base (subterranea-type scales), slightly increasing in size antero-posteriorly (3.4 – 5 µm), covering the dorsal and dorsolateral body. Hydrofoil scales on the ventrolateral body extending from U05 to U70, increasing in length from anterorior (4µm) to posterior (17µm); all pedunculated scales have similar size along the body. First half of ventral side covered by double-edged scales (2.5 µm wide) between the locomotor cirri. On the posterior half of body, 21 pairs of plates arranged in two alternating rows from U31 to U67, increasing in length from anterior (8 µm) to posterior (13 µm). Seventeen pairs of small plates arranged in two parallel rows located between large plates and hydrofoil scales. Furcal basis bear 10 flat scales on the inner margins, overlapping scales proximally.

Ciliature: Several oral bristles per side (1.5-2µm in length). Two pairs of cephalic sensorial tufts per side with cilia varying in length (minimum 2µm maximum 11µm); first pair tufts projecting at anterior margins of the head, a second pair inserting near the tentacles. Two pairs of dorsal sensory bristles per side (4.5 - 7µm); first pair on the neck and second pair at junction between furca basis and adhesive tubes (at U21 and U88, respectively).

Two parallel rows of cirri running from U3 to U28. Two tufts of 4 cirri on the middle trunk U56.

Digestive tract: Terminal mouth (8 µm in diameter), anterior pharynx bulb well developed (8.3 µm). Intestine of similar width throughout (10 µm). Ventral anus at U70.

Remarks: *Xenotrichula* sp. nov. falls within a clade that includes *X. guadelupensis, X. cornuta, X. velox* and all species previously assigned to genus *Heteroxenotrichula* (chapter 1), by sharing the apomorphic feature subterranea-type scales covering the dorsal body. However, *Xenotrichula* sp. nov. can be distinguished from its co-generic species by the presence of only two pairs of sensorial bristles along the body, the two alternating rows of flat plate on the trunk and by the double edge scales on pharynx region on the ventral side.

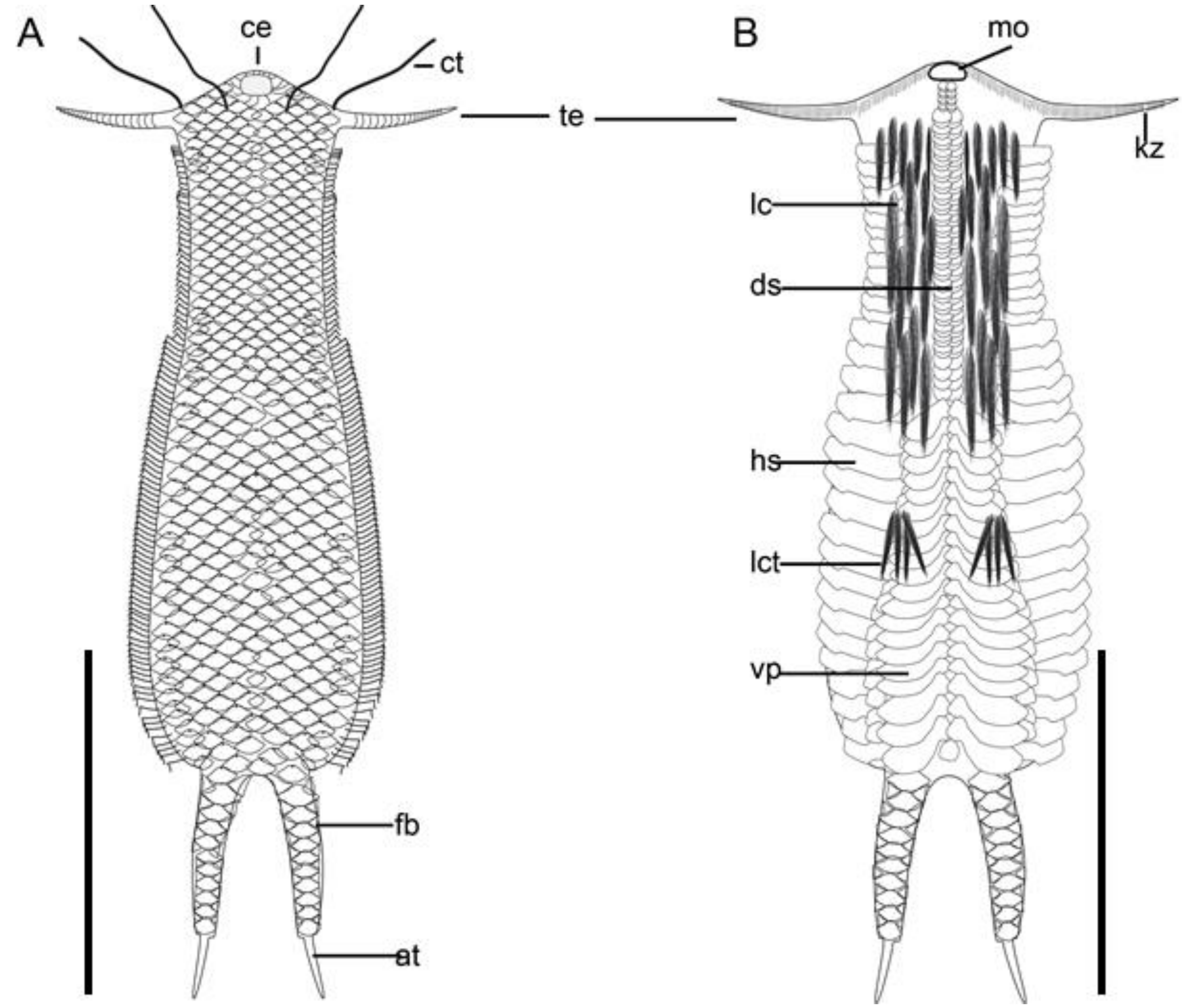

Figure 2 – Schematic draw of *Xenotrichula* sp. nov. A – Dorsal body. B – Ventral Body. Abbreviations: at, adhesive tubes; ce, cephalion; ct, cephalic sensorial tufts; ds, double edge scales; fb, furca base; fs, flat scales at furca base; hs, hydrofoil scales; kz, tranversal row of cilia each side from the mouth extending to the tentacle lenght; lc, locomotory cirri; lct, locomotory cirri tufts; mo, mouth; ps, pedunculated scale; sbi, sensorial bristle insertion; te, tentacle; vp, ventral plates. Scale bar: 50 µm.

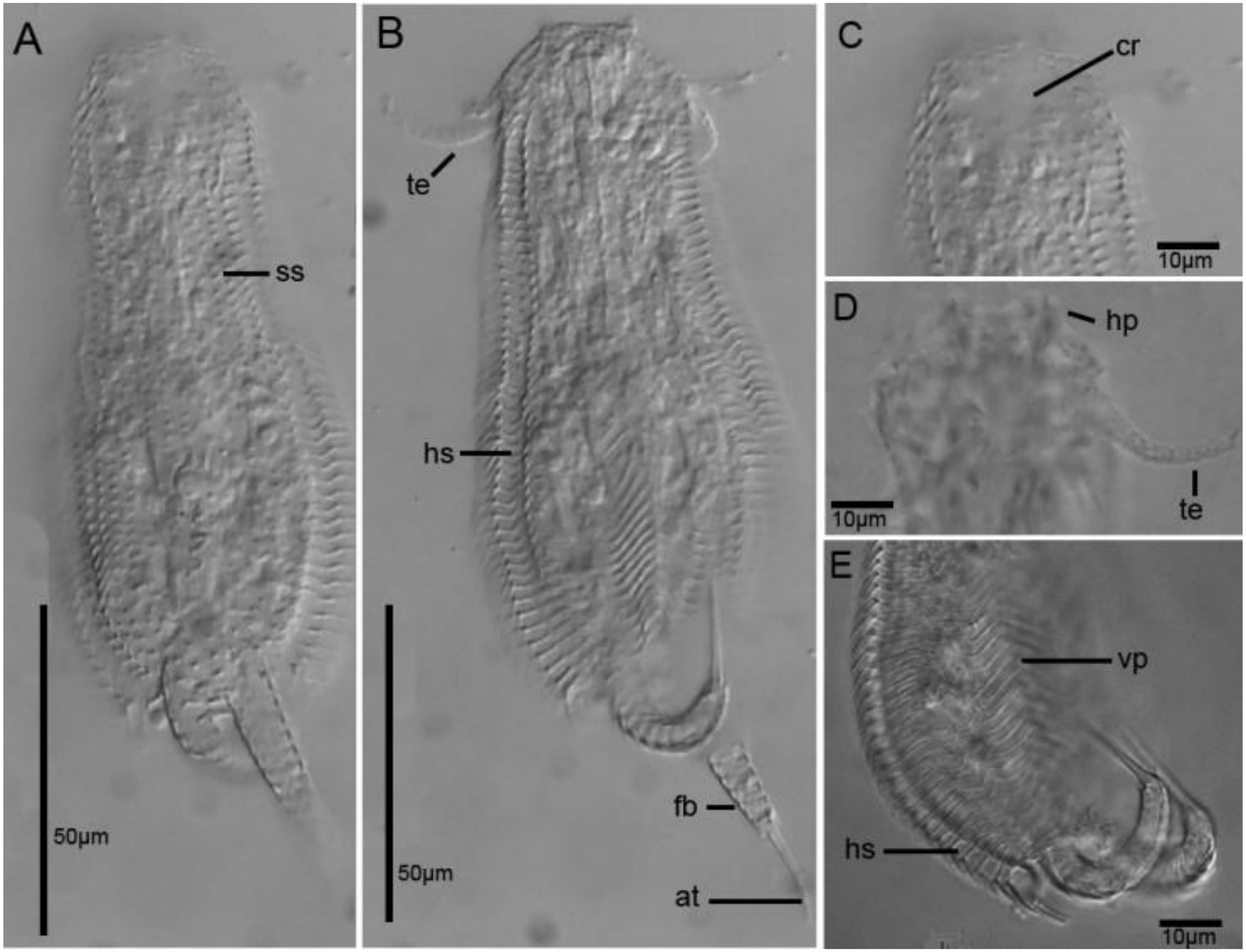

Figure 3 – DIC photomicrographies of *Xenotrichula* sp. nov. A. Dorsal view; B. Ventral view of the body; C. Cephalic region on the dorsal head; D. Close-up on the ventral head; E. Ventrolateral trunk. Scale bars 50 μm Abbreviations: at, adhesive tubes; cr, cephalion region; fb, furca base; hp, hard projection at side of mouth; hs, hydrofoil scales; ss, subterranea-type scale; te, tentacle; vp, ventral plates

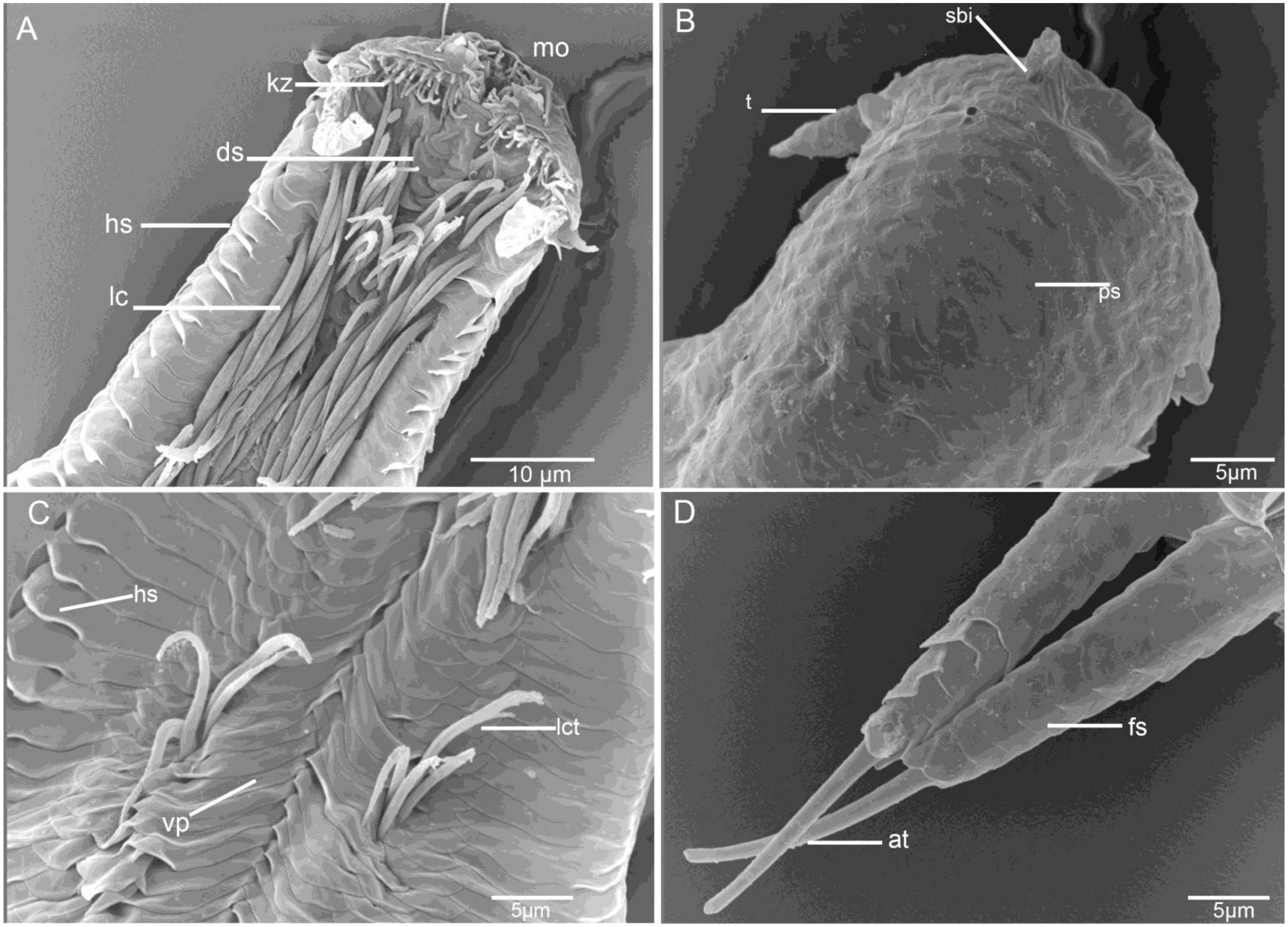

Figure 4 - SEM photos of *Xenotrichula* sp. nov. A. Ventral view of anterior half of body. B. Dorsal view of the head. C. Ventral view of middle trunk. D. Dorsal view of furca. Abbreviations: at, adhesive tubes; ce, cephalion; ds, double edge scales; fs, flat scales at furca appendix; hs, hydrofoil scales; kz, tranversal row of cilia each side of mouth extending to the tentacle lenght; lc, locomotory cirri; lct, locomotory cirri tufts; mo, mouth; ps, pedunculated scale; sbi, sensorial bristle insertion; t, tentacle; vp, ventral plates.

Discussion

For many years, the knowledge of Brazilian marine gastrotrichs was based on the pioneer taxonomical study carried out by Todaro & Rocha (2004, 2005[4]) along the northern coasts of the state of São Paulo (cities of Ubatuba, Caragutatuba, Ilhabela and São Sebastião) and south coast of the state of Rio de Janeiro (city of Paraty). Although the vast majority of Brazilian sandy shores have never been sampled, in the last several years, new species continue to be found on north coast of state of Rio de Janeiro (unpublished data) and the states of Espírito Santo (Araújo et al. 2016) and Bahia (Araujo et al. 2014).

These new records show an interesting panorama regarding the distribution patterns of Brazilian gastrotrichs. The endemic species from Brazil have two clear patterns of latitudinal range of distribution, narrow or wide. In the first case, *Pseudostomella squamalongispinosa* Araujo, Balsamo & Garraffoni, 2014, *Ptychostomella lamelliphora* Todaro, 2013, *Crasiella fonseci* Hochberg, 2014, *Macrodasys fornerise* Todaro & Rocha, 2004 and *Dactylopodola todaroi* Garraffoni, Di Domenico & Hochberg, 2016b are distributed in only a few sandy beaches (sometimes in only one). On the other hand, *Pseudostomella dolichopoda* and *Xenotrichula* sp. nov. have much wider distributions and were found in two distinct biogeographic realms (Tropical Southwestern Atlantic and Warm Temperate Southwestern Atlantic – (Spalding et al. 2007). This pattern with high number of species with narrow distribution and low numbers with wide distribution follows the hypothesis of Garraffoni et al. (2016a) for gastrotrich diversity of the Brazilian southeast coast. These authors pointed out that the gastrotrich biodiversity is best explained by sediment textures, tidal zones and localities, thus, they were not uniformly distributed along the coast.

With regards to the new species, it was found in both low and high energy sandy beaches like many other xenotrichulids (Chapter 1) (Ruppert 1979). It will be interesting to know if future studies of unstudied Brazilian beaches reveal this pattern.

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[4] Todaro (2012, 2013) described the new species *Pseudostomella dolichopoda* and *Ptychostomella lamelliphora*, that Appeared, respectively, as *Pseudostomella* sp. and *Ptychostomella* sp. in Todaro and Rocha (2004; 2005)

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References

Araújo TQ, Atherton S, Hochberg R (2015) First record and a description of a new species of *Oregodasys* (Gastrotricha: Macrodasyida: Thaumastodermatidae) from Tobago. Proc Biol Soc Washingt 128:190–199. doi: 10.2988/0006-324X-128.3.176

Araujo TQ, Balsamo M, Garraffoni ARS (2014) A new species of *Pseudostomodella* (Gastrotricha, Thaumastodermatidae) from Brazil. Mar Biodivers 44:243–248. doi: 10.1007/s12526-013-0196-x

Araújo TQ, Wieloch AH, Vidigal THD, et al (2016) *Pseudostomella dolichopoda* Todaro, 2012 and *P. cataphracta* Ruppert, 1970 (Gastrotricha: Thaumastodermatidae): new records from Brazil and USA and an updated key to the genus. Check List 12:1986. doi: 10.15560/12.6.1986

Balsamo M, D`Hondt J-L, Kisielewski J, et al (2015) Fauna Europaea: Gastrotricha. Biodivers Data J 3:e5800. doi: 10.3897/BDJ.3.e5800

Balsamo M, D`Hondt J-L, Pierboni L, Grilli P (2009) Taxonomic and nomenclatural notes on freshwater Chaetonotida. Zootaxa 2158:1–19.

Balsamo M, Todaro MA (2002) Gastrotricha. In: Rundle SD, Robertson AL, Schmid-Araya JM (eds) Freshwater Meiofauna: Biology and Ecology. Backhuys Publishers, Leiden, pp 45–61

Garraffoni ARS, Di Domenico M, Hochberg R (2016) New records of marine Gastrotricha from São Sebastião Island (Brazil) and the description of a new species. Mar. Biodivers. 1–9.

Gosse PH (1864) The natural history of the hairy-backed animalcules (Chaetonotidae). Intellect Obs 5:387–406.

Hochberg R, Litvaitis MK (2000) Hexamethyldisilazane for scanning electron microscopy of Gastrotricha. Biotech Histochem 75:41–44. doi: 10.3109/10520290009047984

Hummon WD (1974) Some taxonomic revisions and nomenclatural notes concerning marine and brackish-water gastrotricha. Trans Am Microsc Soc 93:194–205.

Hummon WD, Balsamo M, Todaro MA (1992) Italian marine Gastrotricha: I. Six new and one redescribed species of Chaetonotida. Bolletino di Zool 59:499–516. doi: 10.1080/11250009209386711

Kånneby T (2016) Phylum Gastrotricha. In: Thorp and Covich's Freshwater Invertebrates. Elsevier, pp 115–130

Kieneke A, Riemann O, Ahlrichs WH (2008) Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. Zool Scr 37:429–460. doi: 10.1111/j.1463-6409.2008.00334.x

Kieneke A, Schmidt-Rhaesa A, Hochberg R (2015) A new species of Cephalodasys (Gastrotricha, Macrodasyida) from the Caribbean Sea with a determination key to species of the genus. Zootaxa 3947:367. doi: 10.11646/zootaxa.3947.3.4

Kolicka M (2017) New Arctic Gastrotricha — Three *Chaetonotus* (Chaetonotidae) from Longyearbyen (Spitsbergen, Svalbard Archipelago). Ann Zool 67:131–180. doi: 10.3161/00034541ANZ2017.67.1.015

Kolicka M, Dabert M, Dabert J, et al (2016) *Bifidochaetus*, a new Arctic genus of freshwater Chaetonotida (Gastrotricha) from Spitsbergen revealed by an integrative taxonomic approach. Invertebr Syst 30:398. doi: 10.1071/IS16001

Kolicka M, Janikowska E, Kotwicki L (2015) Baltic Sea Gastrotricha—one new species and one new record of Chaetonotida from Poland. Zootaxa 4027:487. doi: 10.11646/zootaxa.4027.4.2

Rao GC, Clausen C (1970) *Planodasys marginalis* gen. et sp. nov. and Planodasyidae fam. nov. (Gastotricha Magrodasyoidea). Sarsia 42:73–82. doi: 10.1080/00364827.1970.10411164

Remane A (1925) Organisation und systematische Stellung der aberranten Gastrotrichen. Zool Anz 1:121–128.

Remane A (1927) *Xenotrichula velox* nov. gen. nov. spec., ein chaetonotoides Gastrotrich mit mäennlichen Geschlectsorganen. Zool Anz 71:289–294.

Ruppert EE (1979) Morphology and systematics of the Xenotrichulidae (Gastrotricha, Chaetonotida). Mikrofauna des Meeresbodens 76:239–292.

Spalding MD, Fox HE, Allen GR, et al (2007) Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. Bioscience 57:573. doi: 10.1641/B570707

Todaro MA (2017) Gastrotricha. http://www.gastrotricha.unimore.it/default.htm. Accessed 1 Jan 2017

Todaro MA, Rocha CEF (2004) Diversity and distribution of marine Gastrotricha along the northern beaches of the state of São Paulo (Brazil), with description of a new species of Macrodasys (Macrodasyida, Macrodasyidae). J Nat Hist 38:1605–1634. doi: 10.1080/0022293031000156169

Todaro MA, Rocha CEF (2005) Further data on marine gastrotrichs from the State of São Paulo and the first records from the State of Rio de Janeiro (Brazil). Meiofauna Mar 14:27–31.

Wilke U (1954) Mediterrane Gastrotrichen. Zool Jahrbücher Abteilung für Syst Ökologie und Geogr der Tiere 82:497–550.