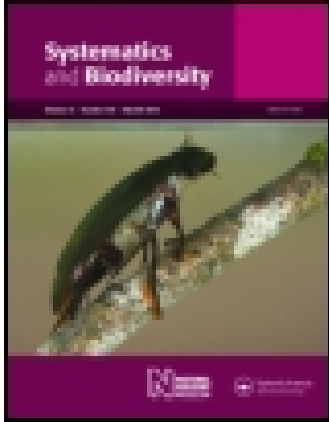


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## Research Article

# A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of Macrotrichida based on molecular data

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Gastrotricha is a phylum of aquatic microinvertebrates counting about 850 species divided into the two orders Chaetonotida and Macrotrichida. Currently, Chaetonotida includes 8 families and 31 genera while Macrotrichida includes 9 families and 32 genera; however, systematics is in a state of flux due to phylogenetic incongruences of the classical systematization. The process of re-systematization will benefit from additional surveys of insufficiently known taxa and especially from the discovery of new species bearing novel characteristics that could help to recognize plesiomorphy in these morphologically diverse animals. Herein, a new and interesting macrotrichidan species from the northwestern shore of Jamaica is described. Specimens up to 1170  $\mu\text{m}$  in length have: a vermiform body with numerous epidermal glands; naked cuticle; head weakly demarcated; posterior end in the form of two caudal pedicles; TbA, arranged in two diagonal rows, inserting directly on the cuticle; TbL/TbVL scarce; TbD and TbV absent; TbP at the tip of each caudal pedicle and along its inner margins; mid-sized terminal mouth; pharynx with pores at the base; PhIJ at U20; intestine rectilinear, anus ventral; hermaphroditic sexual apparatus; gonads paired: female anterior, male posterior; gametes maturing in a caudo-cephalic direction; spermatozoa relatively short, with spiralled head and spiralled tail; sperm ducts directed posteriorly, converging ventrally on the midline and joining the sac-like caudal organ; and frontal organ, dorsal to the intestine, not obviously muscularized. External morphology and layout of the reproductive system appear so unique among Gastrotricha to grant the establishment of a new taxon, for which the name *Hummondasys jamaicensis* gen. et sp. nov. is proposed. Furthermore, to allocate the new genus, the creation of the new family Hummondasyidae is proposed based both on the morphological peculiarities and results of phylogenetic analyses based on the 18S rRNA gene, which involved all of the relevant macrotrichidan taxa.

<http://zoobank.org/urn:lsid:zoobank.org:pub:595575EA-5ADF-4D0C-AD8B-8BC87E3D3905>

**Keywords:** biodiversity, benthos, Caribbean Sea, meiofauna, phylogeny, taxonomy

## Introduction

Gastrotricha is a phylum of aquatic microinvertebrates which includes about 850 species (as in June 2014) divided into the two orders Chaetonotida and Macrotrichida. Chaetonotida includes tenpin-shaped, hermaphroditic or parthenogenetic species found in marine, brackish or freshwater habitats, whereas Macrotrichida includes, as a rule, vermiform, hermaphroditic species living interstitially in the sand of marine ecosystems.

The alpha biodiversity of the entire phylum is in a state of flux as testified by the incessant description of new species (e.g. freshwater: K anneby, 2013; Kolicka *et al.*, 2013;

Suzuki *et al.*, 2013; Todaro *et al.*, 2013; marine: Hummon, 2011; Todaro *et al.*, 2011a; Atherton & Hochberg, 2012a, b; Kieneke *et al.*, 2013a; Lee *et al.*, 2013, Atherton, 2014), whereas cladistics studies of the last decade, challenging the phylogenetic congruence of the classical systematization, have brought about a notable increase in the number of the recognized genera and families (Todaro *et al.*, 2006a, 2012a; Leasi & Todaro, 2008; Hummon & Todaro, 2010). Currently, the order Chaetonotida is subdivided into 8 families and 31 genera while the order Macrotrichida includes 9 families and 32 genera (Todaro, 2013a). However, the effort to make systematization more congruent with the results of phylogenetic studies is far from completed, as best testified by the recent work on the largest family of the phylum (K anneby *et al.*, 2013).

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One of the problems that makes revision difficult is that the anatomical ground patterns of taxa putatively belonging to the different evolutionary lines are still not well-known (e.g. Ruppert, 1991; Hochberg & Litvaitis, 2003; Kieneke *et al.*, 2008; Rothe *et al.*, 2011). If the process of re-systematization will definitely benefit from additional surveys of insufficiently known taxa (see Guidi *et al.*, 2014), the discovery of new species with novel characteristics could help to identify plesiomorphy in these morphologically diverse animals, thus providing a more solid ground for their natural grouping.

Herein, a new interesting macrodasyidan species sampled in 2011 during a survey along the northwestern shores of Jamaica is described. External morphology and traits of the reproductive system of the new species appear so unique among Gastrotricha to grant the creation of a new genus, but at the same time makes the affiliation of these animals to any of the currently recognized families difficult. A discussion (and a solution) in this regard is also provided in the consideration of results of a phylogenetic analysis based on the 18S rRNA gene that included all of the relevant taxa of the Macrodasyida taxonomic spectrum.

The study is part of a larger research programme aimed at shedding light on the diversity and phylogeny of gastrotrich species of the Tropical North-Western Atlantic. From 2010 to 2013, several international groups of researchers surveyed the gastrotrich fauna of different islands in the South Floridian, Bahamian, Lesser Antilles & Central Caribbean ecoregions. Accounts of these and related studies can be found in Hummon (2010), Hochberg & Atherton (2010, 2011), Atherton & Hochberg (2012a, b), Hochberg *et al.* (2013a, b), Kieneke *et al.* (2013a, b) and Atherton (2014). Research teams including the senior author have visited three islands: St. John in the US-Virgin islands, Jamaica and Curaçao. Part of the information and/or taxa found in the surveyed islands appears in several papers (e.g. Hummon *et al.*, 2010; Kanneby *et al.*, 2012, 2013; Todaro *et al.*, 2012a; Todaro & Leasi, 2013).

## Materials and methods

### Sampling and morphological analysis

Sampling campaigns took place in February 2011 and included 10 locations along the North and West coasts of Jamaica. The species described herein was found in sublittoral samples collected at a water depth of 0.8 m, at the northern side of Long Bay beach (Negril, Montego Bay). About 2 litres of sediment were sampled by skin-diving, collected into 500 mL plastic jars (Todaro, 2002) and soon after brought to the field laboratory (Discovery Bay Marine Laboratory). No special permission/permits were needed to collect these animals as gastrotrichs are microscopic, non-pathogenic organisms. Field studies did not involve endangered species and sampling was carried out on a public beach.

In the laboratory, the specimens were extracted daily with the narcotization-decantation technique using a 7% magnesium chloride solution within one week of collection; the supernatant was poured into plastic Petri dishes (3 cm diameter) and scanned for gastrotrichs at a maximum magnification of 50× under a Wild M3 stereomicroscope (Todaro & Hummon, 2008). When located, each individual gastrotrich specimen was mounted on a glass slide and observed *in vivo* with Nomarski differential interference contrast optics using a Zeiss Axio Scope.A1. During observation, the specimens were photographed with a DS-5M Nikon digital camera and measured using the Nikon NIS-F software. Four specimens were fixed in 95% ethanol and stored for DNA analysis. The description of the new species follows the scheme adopted by Hummon *et al.* (1993), whereas the locations of some morphological characteristics along the body are given in percentage units (U) of total body length measured from the anterior to posterior.

Abbreviations are as follows: LT, total body length; PhIJ, pharyngo-intestinal junction; TbA, anterior adhesive tubes; TbD, dorsal adhesive tubes; TbDL, dorsolateral adhesive tubes; TbL, lateral adhesive tubes; TbV, ventral adhesive tubes; TbVL, ventrolateral adhesive tubes; TbP, posterior adhesive tubes.

### Granulometry and ecology

Granulometric analysis of the substrata was carried out according to Todaro *et al.* (2006b). Mean grain size, sorting coefficient, kurtosis, and skewness were calculated by a computerized programme based on the equation of Seward-Thompson & Hails (1973). The rationale for the key to the ecological characteristics of the species, according to Hummon *et al.* (1992), is as follows. The frequency of a species from among a sample series (i.e. frequency of a species in samples collected in any given sampling trip): sparse, found in less than 10% of samples; occasional, found in 10–30% of samples; common, found in 30–60% of samples; and usual, found in more than 60% of samples. Abundance of a species among other species of a sample: rare, less than 1% of a sample; scarce, 3–5% of a sample; numerous: 10–20% of a sample (often a sub-dominant); and prevalent, more than 30% of a sample (usually dominant or co-dominant).

### Molecular analysis

To estimate the phylogenetic relationships of the new taxa within the order Macrodasyida, the almost complete 18S rRNA gene sequences of 43 species (44 specimens) belonging to 23 genera within the nine currently recognized families were used (Table 1). A representative of the order Chaetonotida, *Xenotrichula intermedia* (Xenotrichulidae), was chosen as the out-group in the analyses. The sequences used are the same as in Todaro *et al.* (2012a). With regard

**Table 1.** Gastrotrich taxa involved in the molecular analyses. Origin, reference and GenBank accession number are given.

Taxon	Origin	Reference	Accession
<b>Cephalodasyidae</b>			
<i>Cephalodasys</i> sp.	White Sea, Russia	Petrov <i>et al.</i> (2007)	AY963691
<i>Dolichodasys</i> sp.	San Isidoro, Italy	Todaro <i>et al.</i> (2003)	AM231778
<i>Mesodasys laticaudatus</i>	Albinia, Italy	Todaro <i>et al.</i> (2011b)	JF357657
<i>Mesodasys littoralis</i>	Bou Fichta, Tunisia	Todaro <i>et al.</i> (2011b)	JF357658
<i>Paradasys</i> sp.	Ionian Sea, Italy	Todaro <i>et al.</i> (2003)	AM231781
<i>Pleurodasys helgolandicus</i>	Ibiza, Spain	Todaro <i>et al.</i> (2012a)	JN203486
<b>Dactylopodolidae</b>			
<i>Dactylopodola</i> cf. <i>baltica</i>	Ras Alard, Kuwait	Todaro <i>et al.</i> (2011b)	JF357650
<i>Dactylopodola mesotyphle</i>	Punta Ala, Italy	Todaro <i>et al.</i> (2011b)	JF357651
<i>Dactylopodola typhle</i>	Bou Fichta, Tunisia	Todaro <i>et al.</i> (2011b)	JF357652
<i>Dactylopodola typhle</i>	Torre Civette, Italy	Todaro <i>et al.</i> (2011b)	JF357653
<b>Hummondasyidae</b>			
<i>Hummondasys jamaicensis</i>	Negril, Jamaica	Present study	KM083602
<b>Lepidodasyidae</b>			
<i>Lepidodasys unicarenatus</i>	Pianosa, Italy	Todaro <i>et al.</i> (2011b)	JF357665
<b>Macrodasysidae</b>			
<i>Macrodasys</i> sp. 1	Torre Civette, Italy	Todaro <i>et al.</i> (2011b)	JF357654
<i>Macrodasys</i> sp. 2	Bohuslän, Sweden	Todaro <i>et al.</i> (2011b)	JF357670
<i>Urodasys</i> sp.	NA	Giribet <i>et al.</i> (2004)	AY218102
<i>Urodasys</i> sp.1	Florida, USA	Sørensen <i>et al.</i> (2006)	DQ079912
<b>Planodasyidae</b>			
<i>Crsiella</i> sp.	Ilha Bela, Brazil	Todaro <i>et al.</i> (2012a)	JN203488
<i>Megadasys</i> sp.	Grotta del Ciolo, Italy	Todaro <i>et al.</i> (2011b)	JF357655
<i>Megadasys</i> sp. 1	Porto Cesareo, Italy	Todaro <i>et al.</i> (2011b)	JF357656
<b>Redudasyidae</b>			
<i>Anandrodasys agadasys</i>	St. John Island, USA	Todaro <i>et al.</i> (2012a)	JN203487
<i>Redudasys fornerise</i>	Represa do Broa, Brazil	Todaro <i>et al.</i> (2012a)	JN203489
<b>Thumastodermatidae</b>			
<i>Acanthodasys</i> sp. a	Capraia, Italy	Todaro <i>et al.</i> (2011b)	JF357638
<i>Acanthodasys aculeatus</i>	Capraia, Italy	Todaro <i>et al.</i> (2011b)	JF357639
<i>Diplodasys ankei</i>	Meloria, Italy	Todaro <i>et al.</i> (2011b)	JF357624
<i>Diplodasys meloriae</i>	Meloria, Italy	Todaro <i>et al.</i> (2011b)	JF357640
<i>Oregodasys ocellatus</i>	Meloria, Italy	Todaro <i>et al.</i> (2011b)	JF357642
<i>Oregodasys ruber</i>	Meloria, Italy	Todaro <i>et al.</i> (2011b)	JF357625
<i>Oregodasys tentaculatus</i>	Meloria, Italy	Todaro <i>et al.</i> (2011b)	JF357626
<i>Pseudostomella etrusca</i>	Albinia, Italy	Todaro <i>et al.</i> (2011b)	JF357633
<i>Ptychostomella</i> sp. 1	Ilha Bela, Brazil	Todaro <i>et al.</i> (2011b)	JF357643
<i>Ptychostomella tyrrhenica</i>	Albinia, Italy	Todaro <i>et al.</i> (2011b)	JF357634
<i>Tetranchyroderma papii</i>	Sardegna, Italy	Todaro <i>et al.</i> (2011b)	JF357637
<i>Tetranchyroderma esarabdophorum</i>	Mahdia, Tunisia	Todaro <i>et al.</i> (2011b)	JF357627
<i>Tetranchyroderma hirtum</i>	Capraia, Italy	Todaro <i>et al.</i> (2011b)	JF357628
<i>Tetranchyroderma thysanophorum</i>	Albinia, Italy	Todaro <i>et al.</i> (2011b)	JF357630
<i>Thaumastoderma moebjergi</i>	Bohuslän, Sweden	Todaro <i>et al.</i> (2011b)	JF357671
<i>Thaumastoderma ramuliferum</i>	Meloria, Italy	Todaro <i>et al.</i> (2011b)	JF357631
<b>Turbanellidae</b>			
<i>Paraturbanella dohrni</i>	Punta Ala, Italy	Todaro <i>et al.</i> (2011b)	JF357659
<i>Paraturbanella pallida</i>	Capraia, Italy	Todaro <i>et al.</i> (2011b)	JF357660
<i>Paraturbanella teissieri</i>	Punta Ala, Italy	Todaro <i>et al.</i> (2011b)	JF357661
<i>Turbanella bocqueti</i>	Tramore, Ireland	Todaro <i>et al.</i> (2011b)	JF357662
<i>Turbanella cornuta</i>	Chioggia, Italy	Todaro <i>et al.</i> (2011b)	JF357663
<i>Turbanella lutheri</i>	Torö, Sweden	Todaro <i>et al.</i> (2011b)	JF357669

(continued)

**Table 1.** (Continued)

Taxon	Origin	Reference	Accession
<b>Xenodasyidae</b>			
<i>Xenodasys riedli</i>	St. John Island, USA	Todaro <i>et al.</i> (2012a)	JN203490
<b>Xenotrichulidae*</b>			
<i>Xenotrichula intermedia</i>	Mahdia, Tunisia	Todaro <i>et al.</i> (2011b)	JF357664

\* Order Chaetonotida; NA, data not available.

to the new taxon, DNA was extracted from a single, whole specimen using the QIAamp DNA mini kit (QIAGEN), with columns from the QIAamp DNA micro kit (QIAGEN), according to the manufacturer's instructions. The extract was then used as a template for the subsequent amplifications. A fragment of DNA of approximately 1700 bp was amplified using the 0.2 mL PuReTaq Ready-To-Go PCR beads (GE Healthcare). For amplification, 0.5 mL of each primer, 2 mL of DNA and 22 mL of purified water were assembled in the RTG-PCR tubes, yielding a final volume of 25 mL. Primer sequences and PCR programs were the same as in Todaro *et al.* (2011b), with the polymerase chain reactions carried out in a Biometra personal thermocycler. The PCR-products were purified using the QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions and sent for sequencing to Macrogen, Korea ([www.macrogen.co.kr](http://www.macrogen.co.kr)). Contigs were assembled using Staden v 1.6.0 (Staden, 1996). The 45 sequences were aligned with MUSCLE (Multiple sequence comparison by Log-Expectation), as implemented in MEGA 5.2 (Tamura *et al.*, 2011), using the default parameters. The dataset, which consisted of 1890 nucleotide characters, was subsequently converted into both interleaved Nexus and Fasta formatted files and analysed phylogenetically using three different approaches: (i) Maximum Parsimony (MP), (ii) Maximum Likelihood (ML) and (iii) Bayesian inference (MrBayes 3.1.2) (Ronquist & Huelsenbeck, 2003). For the analyses carried out with MrBayes and ML, the evolutionary model of nucleotide substitution GTR+G+I was used, which is favoured by both the AICc and the lnL criteria in MrModeltest v2.3 (Nylander, 2004) and Mega 5.2. For the Bayesian analysis, two trials with four simultaneous chains were run for 6 000 000 generations; trees were sampled every 100th generation after a burn-in of 15 000 generations. A 50% consensus tree was produced with TreeView (Page, 1996). For both the ML and MP analyses, the 'use-all sites' data treatment option was selected, with the phylogeny test set to bootstrap with 1000 replications.

## Results

Order Macrotrichida Remane, 1925 [Rao & Clausen, 1970]

Family Hummondasyidae fam. nov.

<http://zoobank.org/urn:lsid:zoobank.org:act:5E8E2032-00E2-4A5C-A0F8-8662DB4BC20C>

**Diagnosis.** Same as the genus.

Genus *Hummondasyis* gen. nov.

<http://zoobank.org/urn:lsid:zoobank.org:act:7DDBCC46-52F7-4443-9B06-AFBB4C1DB040>

**Diagnosis.** Body elongate, up to 1170  $\mu\text{m}$  in length, and rather narrow, up to 68  $\mu\text{m}$  in width, flattened ventrally and vaulted dorsally, with numerous epidermal glands. Cuticular covering smooth, deprived of scales and/or spines. Head weakly demarcated, showing a slight swelling on each side and sparse sensory cilia but neither piston pits nor eye spots. Posterior region in the form of two caudal pedicles. Sensory hairs arranged singly in lateral and dorsolateral columns along the body and sparsely on the dorsal and lateral sides of the head. Ventral locomotor ciliation in the form of a continuous field under the head and along most of the pharyngeal region, thereafter arranged in two narrow longitudinal bands that run separately for 2/3 of the trunk and then merge again medially to form loose tufts. TbA, 3 per side, forming a diagonal row on each side, inserting directly on the body surface; TbV, absent; TbVL up to 10 per side; 2 along the pharyngeal region and the remaining along the trunk region; TbD, absent; TbDL, 1 per side in the posterior trunk region; TbP, 4 per side, 2 at the end of each caudal pedicle and 2 along the inner side of each pedicle. Mouth terminal, of mid-size (9  $\mu\text{m}$  in diameter), leading to a small buccal cavity (10  $\mu\text{m}$  in length), which opens into a 178  $\mu\text{m}$  long and 10–12  $\mu\text{m}$  wide pharynx; pharyngeal pores near the base with ventrolateral openings. Pharyngeo-intestinal junction at about U20. Intestine increases in width from the PhIJ to mid-body and gradually narrows up to the posterior body end; anus ventral at U96.

Hermaphroditic, with paired gonads, female anterior and male posterior; gametes maturing in a caudo-cephalic direction. Testes, roughly ovoidal in shape with the anterior-most portion at about U70; sperm ducts relatively short, extending posteriorly to about U87 where they fuse on the mid-ventral plane joining internally the caudal organ. Mature sperm are short, spiralled cells, 20–22  $\mu\text{m}$  length, composed of two recognizable regions: a 6–8  $\mu\text{m}$  long head and 14–16  $\mu\text{m}$  tail. Sperm may be seen as single cells or arranged tightly side-by-side to form discrete clusters of 12–20 sperm each. Frontal organ dorsal to the

intestine, centred at U81; bulky, roughly ovoid in shape with wall apparently not muscularized, up to 78  $\mu\text{m}$  in length and 39  $\mu\text{m}$  in width, not subdivided into spermatheca and seminal receptacle regions but usually uniformly filled with sperm clusters and refringent globules; the internal pore is at the frontal end; an external pore for the intake of the allosperm is likely to be present but so far has not been detected. Caudal organ, ventral to the intestine, centred at U88; relatively small, roughly sac-like in shape with wall apparently not muscularized, up to 52  $\mu\text{m}$  in length and 33  $\mu\text{m}$  in width; glandular material present especially on the posterior region, sperm clusters in the anterior region, when seen. Ovaries anterior to the testes with early oocytes on the lateral sides and largest egg dorsal to the intestine at about mid-body.

**Etymology.** The genus is named after William D. Hummon, ‘maestro’, colleague and friend, in recognition of the remarkable impact of his studies on marine Gastrotricha. Besides contributing substantially to our understanding of the biodiversity, biology and ecology of these animals, first-hand observations of fauna from key areas of the world have allowed Bill Hummon to disentangle many problems concerning species identification, making current taxonomic research much easier. The suffix ‘-dasys’ is traditionally used in most genera of macrodasyidan gastrotrichs and alludes to their dense ciliation.

***Hummondasys jamaicensis* sp. nov.**

<http://zoobank.org/urn:lsid:zoobank.org:act:17DB8C2A-1D8B-4AC1-98A2-2A2094BF2365>

(Figs 1–15)

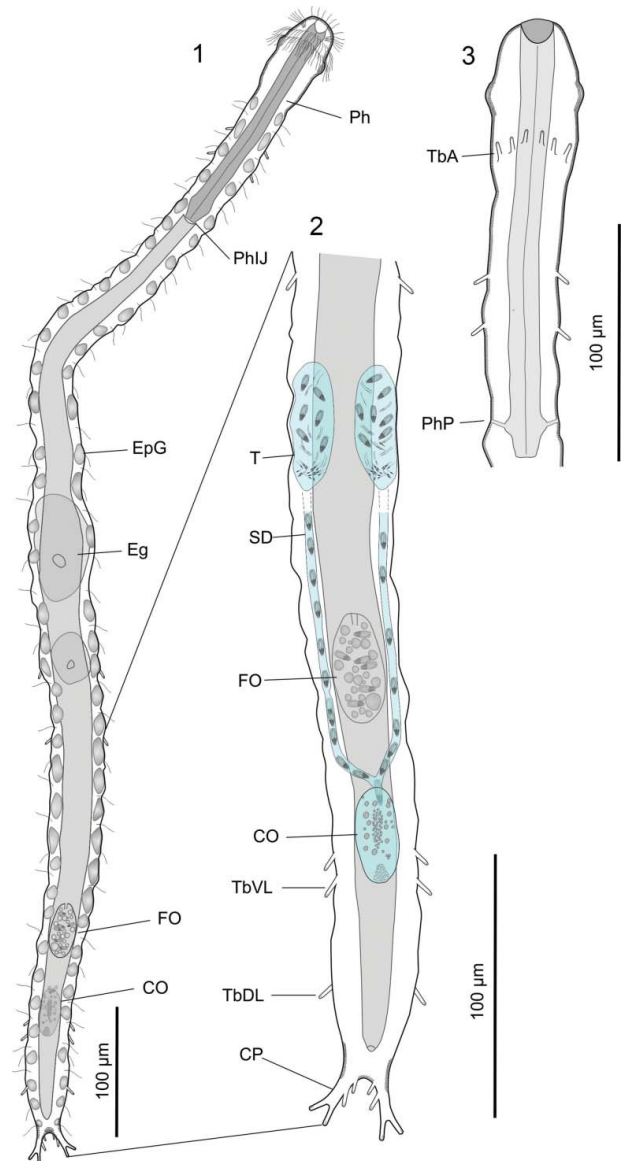
**Diagnosis.** Same as the genus.

**Etymology.** The specific name alludes to the country where the species was first found.

**Examined material.** The description of *Hummondasys jamaicensis* n. sp. is derived from four specimens, three adults and a single juvenile, all collected from the same location. The holotype, LT = 955  $\mu\text{m}$ , is the adult shown in Fig. 4 (International Code of Zoological Nomenclature, Articles 73.1.1, 73.1.4), after observation it was fixed in 95% ethanol and later used for DNA analysis (GenBank accession number KM083602). The juvenile and the two additional studied adults are no longer extant. Three further identified specimens were fixed in alcohol and are kept in the author’s collection.

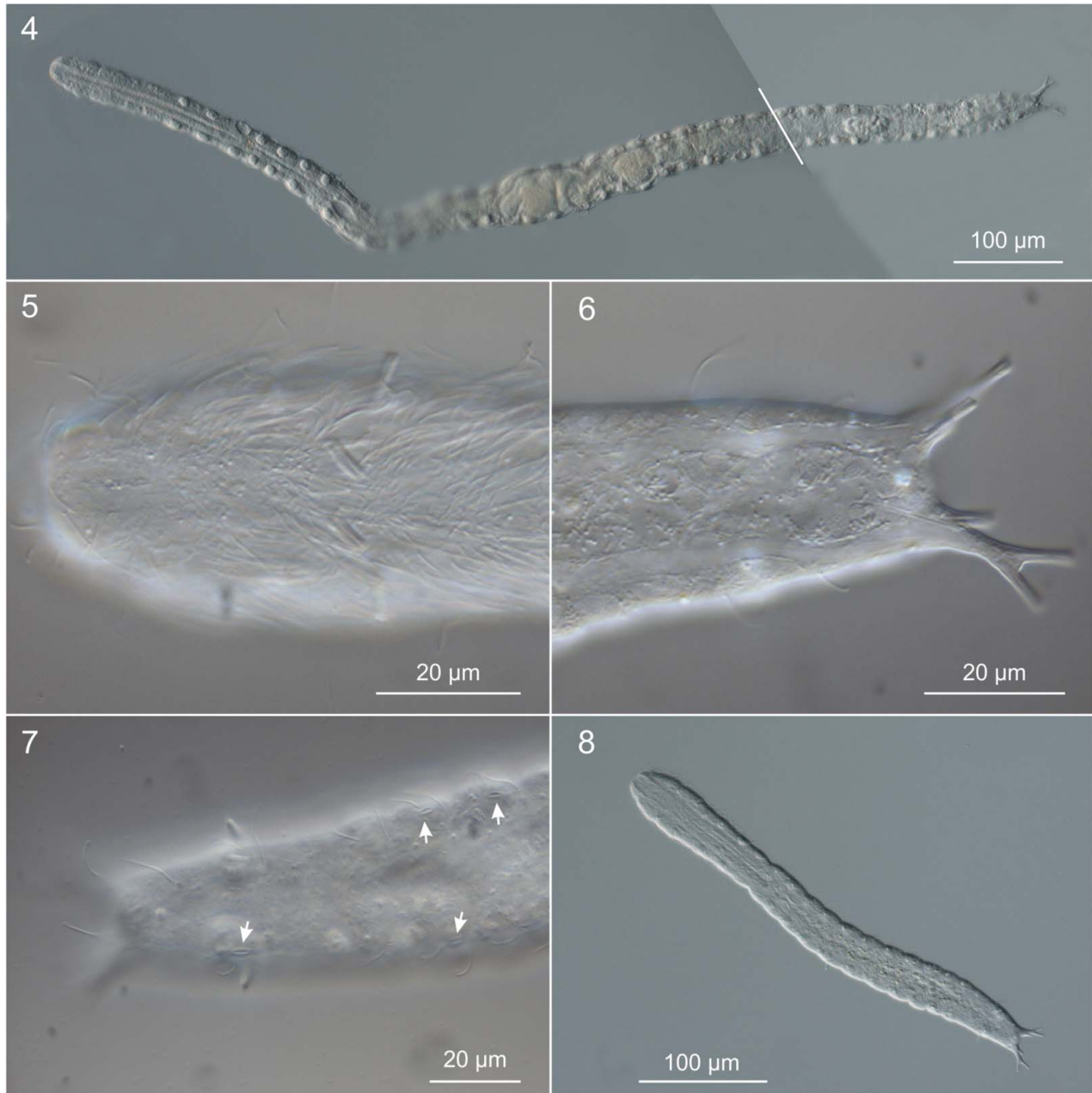
**Type locality.** The sediment samples were collected on 21 February 2011 from Long Bay Beach, Negril, Montego Bay, Jamaica (Lat. 18°19'34" N; Long. 78°20'11" W).

**Ecology.** Common in frequency of occurrence (30–60% of samples), numerous in abundance (10–20% of a



**Figs. 1–3.** Line art illustrations of *Hummondasys jamaicensis* gen. et sp. nov. **1**, habitus as seen from the dorsal side; **2**, posterior trunk region, showing the internal anatomy with the male reproductive structures and the frontal organ; **3**, anterior region, showing the arrangement of the anterior adhesive tubules and the pharyngeal pores. CO, caudal organ; CP, caudal pedicle; FO, frontal organ; Eg, egg; EpG, epidermal gland; Ph, pharynx; PhIJ, pharyngeo-intestinal junction; PhP, pharyngeal pores; SD, sperm duct; T, testis; TbA, anterior adhesive tube; TbDL, dorsolateral adhesive tube; TbVL, ventrolateral adhesive tube. Drawings are made mostly from the holotypic specimen.

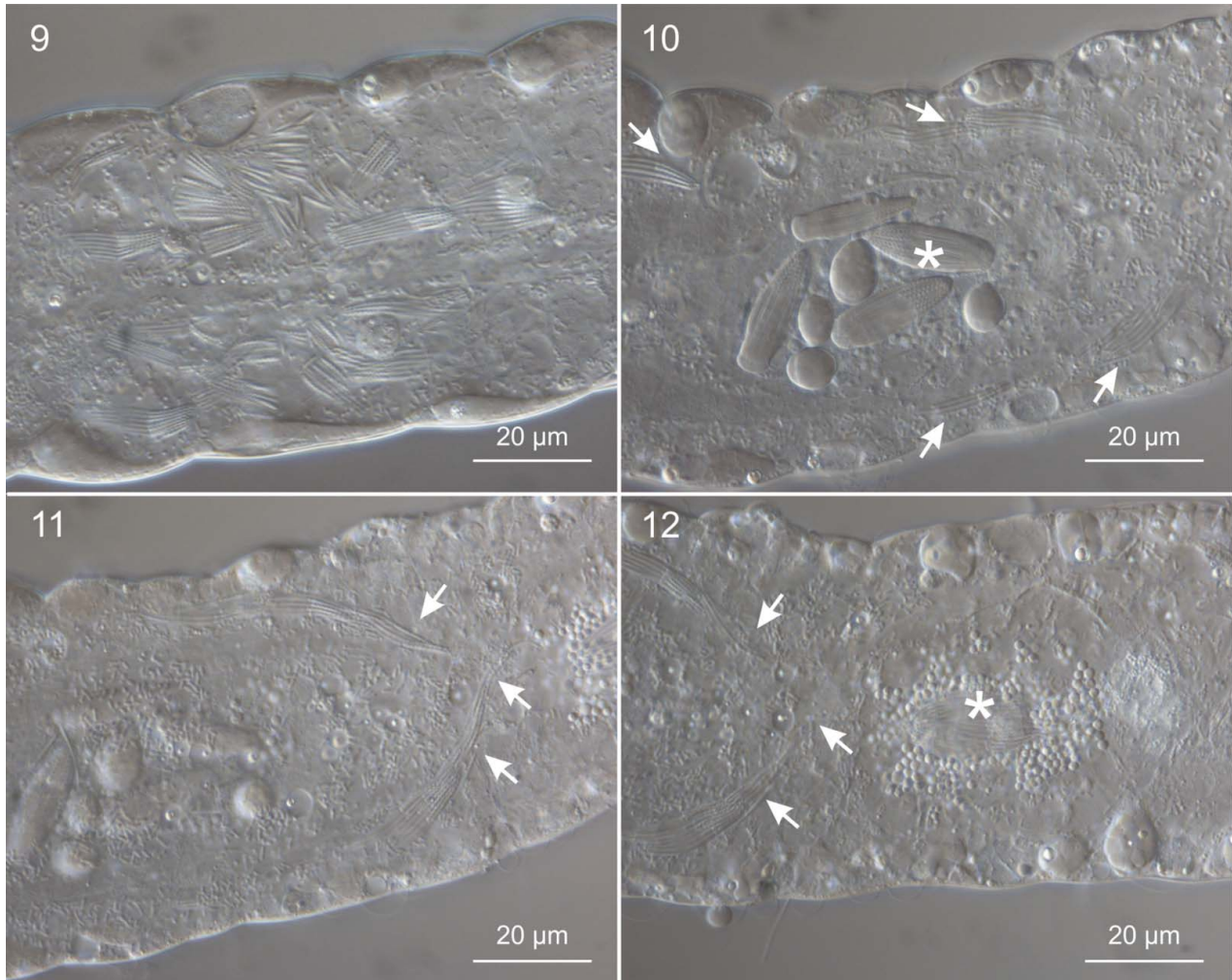
sample); sub-littoral at a water depth of 0.8 m in fine sand (2.4 phi), moderately sorted (0.89 phi) carbonate sand (kurtosis = 2.85; skewness = -0.43). Values of salinity and temperature of the interstitial water at the time of sampling were 34‰ and 26 °C respectively.



**Figs. 4–8.** Differential interference contrast photomicrographs, showing the external morphology of *Hummondasys jamaicensis* gen. et sp. nov. **4**, habitus as seen from the dorsal side; **5**, close-up of the anterior region, ventral view, showing the anterior adhesive tubes; **6**, close-up of the posterior region, ventral view, showing the caudal pedicles; **7**, close-up of the posterior region, dorsal view showing the opening of the epidermal glands (arrows); **8**, habitus of a juvenile. Except for the last, all images refer to the holotypic specimen.

**Description.** Based mostly on the adult holotypic specimens, with a total body length of 955  $\mu\text{m}$ . Body elongate and rather narrow, flattened ventrally and vaulted dorsally, with gently undulating sides due to the presence of numerous epidermal glands; cuticular covering smooth, deprived of scales and/or spines (Figs 1, 4). Body attaining the maximum width at its middle, thereafter gradually narrowing to

the caudal base then broadening again to form two noticeable caudal pedicles that indent medially to U98 (Figs 1, 4, 6, 8). Head weakly demarcated, showing a slight swelling on each side at U03; it bears sparse sensory cilia but neither piston pits nor eye spots are present (Figs 1, 3, 4). Widths of head\pharyngeal region\trunk\caudal base 40\34\50\17  $\mu\text{m}$  at U3.5\U12.5\U51\U97, respectively.



**Figs. 9–12.** Differential interference contrast photomicrographs, showing details of the reproductive traits of *Hummondasys jamaicensis* gen. et sp. nov. **9**, testes with sparse clusters of sperm inside; **10**, same specimen showing discrete clusters of sperm transiting inside the sperm ducts (arrows) and inside the frontal organ (asterisk); **11**, same specimen at a different focal plane showing the merging of the sperm ducts (arrows); **12**, same specimen, merging of the sperm ducts (arrows) and a large cluster of sperm inside the caudal organ (asterisk). Images refer to the largest studied specimen (see text for details).

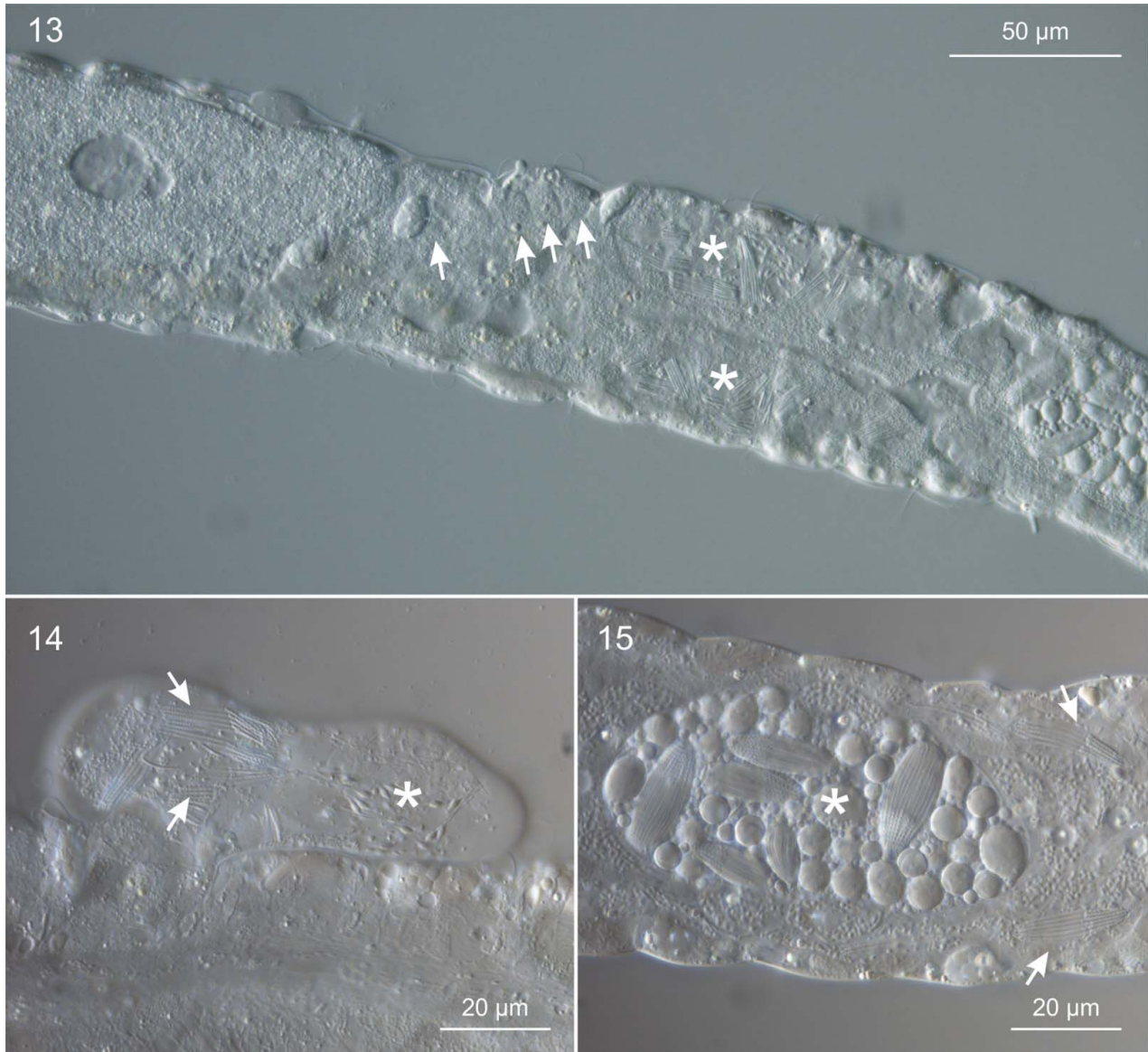
**Epidermal glands:** up to 40 pairs of noticeable epidermal glands are regularly spaced along the pharyngeal and intestinal region from U08 to U97; glands are variable in shape, from round to elliptical, and range in size from 7–9  $\mu\text{m}$  in width to 7–18  $\mu\text{m}$  in length; each gland opens to the exterior via a well-structured pore visible on the dorsal side (Fig. 7). An additional gland is positioned medially in correspondence to the indentation of the caudal pedicle (Fig. 1).

**Ciliation:** Sensory hairs, up to 16  $\mu\text{m}$  in length, arranged in lateral and dorsolateral columns that are regularly spaced along the body and others, 9–14  $\mu\text{m}$  in length, more densely packed on the dorsal and lateral sides of the head (Fig. 1). The ventral locomotor ciliation is not particularly dense, it appears in the form of a

continuous field under the head (Fig. 5) and along most of the pharyngeal region (U01–U17); thereafter, cilia appear arranged in two narrow, longitudinal bands that extend separately for 2/3 of the trunk (U68) and then merge medially to form separate, loose tufts under most of the terminal trunk region (U94). Individual cilia are 10–15  $\mu\text{m}$  in length.

**Adhesive tubes:** TbA, 3 per side, 4–7  $\mu\text{m}$  long, forming a diagonal row on each side, and inserting directly on the body surface, at a certain distance from the post-oral region from U5.5 to U6.5 (Figs 3, 5); TbV, absent; TbVL 6 per side, 4–8  $\mu\text{m}$  long, two along the pharyngeal region at U11 and U14, and four along the second half of the trunk region at U61, U66, U89 and U9,1 respectively (Figs 1, 3); TbD, absent; TbDL, 1 per side (6  $\mu\text{m}$  long) in the posterior





**Figs. 13–15.** Differential interference contrast photomicrographs, showing details of the reproductive traits of *Hammondasys jamaicensis* gen. et sp. nov. **13**, region of the trunk showing the spatial relationship between the female and male gonads; arrows indicate early oocytes whereas the asterisks indicate the testes, **14**, right testis extruded from the body, showing mature sperm in the anterior region (arrows) and maturing sperm in the posterior region (asterisk); **15**, frontal organ (asterisk) containing several sperm clusters and merging sperm ducts (arrows). Images refer to the third studied adult specimen (see text for details).

trunk region at U95 (Figs 1, 2); TbP, 4 per side (4–8  $\mu\text{m}$  long), 2 at the end of each caudal pedicle and 2 along the inner margin of the pedicle (Figs 1, 2, 4, 6).

Digestive tract: Mouth terminal, of mid-size (9  $\mu\text{m}$  in diameter), leading to a small buccal cavity (10  $\mu\text{m}$  in length), which opens into a 178  $\mu\text{m}$  long and 10–12  $\mu\text{m}$  wide pharynx; pharyngeal pores near the base, which open ventrolaterally at U18 (Fig. 3); PhIJ at U19.5. Intestine increases in width from the PhIJ to about mid-body (U51), and gradually narrows up to the posterior body end; anus ventral at U96 (Fig. 1).

Reproductive tract: hermaphroditic; sperm-laden testes, roughly ovoidal in shape with the anterior-most portion at about U70; sperm ducts are relatively short and rectilinear for the most part; they presumably originate at mid-testis and extend posteriorly to about U87 where they fuse on the mid-ventral plane and join internally the caudal organ (Figs 2, 9–12). Mature sperm are short, spiralled cells, 20–22  $\mu\text{m}$  in length, composed of two recognizable regions, a 6–8  $\mu\text{m}$  long head and 14–16  $\mu\text{m}$  tail. Sperm may be seen as single cells or arranged tightly side-by-side to form discrete clusters of 12–20 sperm each. Single

spermatozoa may be seen in the testes and occasionally in the frontal organ, while the discrete clusters are present in the testes, along the sperm duct and inside the caudal and frontal organs (Figs 2, 9–12). Frontal organ dorsal to the intestine (Figs 1, 2, 10, 15), centred at U81; bulky, roughly ovoidal in shape with wall apparently not muscularized, about 42  $\mu\text{m}$  long and 22  $\mu\text{m}$  wide, without a clear anatomical-functional compartmentalization (i.e. not subdivided into spermatheca and seminal receptacle regions); an internal pore is present at the frontal end; an external pore for the intake of the allosperm was not seen. The frontal organ of the holotypic specimen contained two clusters of sperm together with six globular masses of refringent material. Caudal organ ventral to the intestine, centred at U88; relatively small, roughly sac-like in shape with wall apparently not muscularized, about 37  $\mu\text{m}$  long and 17  $\mu\text{m}$  wide (Figs 1, 2, 12); the caudal organ of the holotype contained a cluster of sperm at its frontal end and numerous small globules of secretory material at the caudal end. In the holotype, the ovaries per se were not seen; however, two eggs, dorsal to the intestine, maturing and increasing in size, were present anteriorly (Figs 1, 4). The largest one, 54  $\mu\text{m}$  long and 41  $\mu\text{m}$  wide, was centred at U50 and the other, 40  $\mu\text{m}$  long  $\times$  19  $\mu\text{m}$  wide, at U59.

**Variability and remarks.** The largest adult species attained a total body length of 1170  $\mu\text{m}$  and a maximum body width of 68  $\mu\text{m}$ . Number and arrangement of TbA, TbP and TbDL matched those of the holotype, whereas the number of the TbVL was slightly higher (10 vs. 6 per side) and moreover, the implantation of some of them (e.g. at mid-body) seemed to be more lateral than ventrolateral. In this regard, it should be emphasized that because of the very elongate and flexible body, it is very difficult to position these animals on the slide in a perfect dorso-ventral orientation; consequently, it may be problematic to obtain precise accounts of the adhesive tubes belonging to the different series (e.g. TbVL, TbL and TbDL). The frontal organ was 68  $\mu\text{m}$  long and 38  $\mu\text{m}$  wide and contained four clusters of sperm along with four globular masses of refringent material (Fig. 10). The globular masses are interpreted as residues of the secretory material associated with the sperm cluster entering the frontal organ and likely produced by the caudal organ to facilitate the process of sperm transfer and/or sperm functioning. The caudal organ of this animal was 53  $\mu\text{m}$  long and 34  $\mu\text{m}$  wide (Fig. 12); it contained a very large cluster of sperm (very likely resulting from the union of two distinct clusters) located almost at the centre of the organ and surrounded by secretory material, which appeared to be most abundant in the posterior region (Fig. 12).

The third measured adult was 1023  $\mu\text{m}$  in total body length and attained a maximum body width of 66  $\mu\text{m}$ . Number and arrangement of TbA and TbP matched those of the other two studied adults; unfortunately, a reliable

account of the number and arrangement of tubes of the other series cannot be provided due to the twisted orientation of the specimen on the slide. A large egg was visible at about U56; it was followed by four developing oocytes, all located on the right side with the smallest egg just anterior to the testis yet separated from it (Fig. 13). This observation has particular relevance. Among gastrotrichs, the ovary is known to be either paired and lateral or unpaired and dorsal; consequently, the position of the developing oocytes on the right side of the body in the examined specimen strongly suggests that the ovary is paired and lateral in this species, although a left ovary has not explicitly been observed. The frontal organ of this specimen was 78  $\mu\text{m}$  long and 39  $\mu\text{m}$  wide, and contained six clusters of sperm along with numerous (>15) round globular masses of refringent material (Fig. 15). The caudal organ measured 52  $\mu\text{m}$  in length and 33  $\mu\text{m}$  in width; it showed two clusters of sperm entering from its frontal opening and sparse secretory material inside. Close to the end of the observation, the compression of the specimen between slide and coverslip caused the expulsion of the right testis from the body (Fig. 14) revealing a feature overlooked in the other observed adults: i.e. presence in the testes of a different type of cells in addition to the spiralled spermatozoa described above. This novel cell type is characterized by a spindle-shaped head (3.5  $\mu\text{m}$  in length) and a filiform tail (7  $\mu\text{m}$ ). The cells were located in the posterior portion of the testis, in contrast with the spiralled spermatozoa, which were located in the frontal portion of the gonad. In accordance with the spermatogenesis process described for other species (e.g. Guidi *et al.*, 2004, 2011), these cells are interpreted as precocious stages of spermatozoa. Consequently, it may be said (even without electron microscopy observations) that in this species, the male cells mature in a caudo-cephalic direction, as usually happens in Gastrotricha. The presence of early stages of spermatozoa in the posterior region of the testis makes it unrealistic to hypothesize that the sperm ducts begin at the posterior end of the gonad, as could be seen from light microscopy; instead, they probably originate from the middle of each testis, as recently ascertained under transmission electron microscopy for other taxa e.g. *Dinodasys mirabilis* (Fam. Turbanellidae, see Todaro *et al.*, 2012b) and *Megadasys sterreri* (Fam. Planodasyidae, see Guidi *et al.* 2014).

The measured juvenile specimen reached 385  $\mu\text{m}$  in total length with PhIJ at U34. It had 3 TbA, 2 TbVL and 1 TbDL per side; in addition, each caudal pedicle had 2 tubes at the distal end and 1 tube along the inner margin (Fig. 8). None of the reproductive structures were present.

The metric and meristic information reported above testify to the variability of some traits and highlight the fact that variations are only in part related to the specimen size; for example, the size of the accessory sexual organs depends chiefly on their actual functioning (i.e. what they contain) and only secondarily on the size of the specimens

that bear them. Likewise, a warning is made on the poor taxonomic value (e.g. species identification) of traits whose number and arrangement may be variable and/or difficult to ascertain, as in this case, the adhesive tubes of the lateral series; unless their number exceeds by far the reported statistics.

### DNA-based phylogenetic analysis

The final dataset included 1890 alignable positions, 978 of which are constant and 696 parsimony-informative. The three phylogenetic analyses, carried out with ML, MP and Bayesian approaches, yielded topologies that were highly congruent with each other, with most of the many groups that are in common bearing high nodal support: i.e. bootstrap and Bayesian posterior probability values >72 and 98%, respectively (Figs 16–18). Among the robustly supported groups are (1) the densely sampled families Thaumastodermatidae and Turbanellidae and their recognized subgroupings; (2) the recently highlighted alliance between *Redudasys fornerise* Kisielowski, 1987 and *Anandrodasys agadasys* (Hochberg, 2003); and (3) the sister-group relationship between *Crasiella* Clausen, 1968 and *Megadasys* Schmidt, 1974.

In contrast, the currently recognized Macrodasysidae and Cephalodasyidae never appear as monophyletic due to the scattering along the evolutionary tree of their respective species and/or the alliances between members of different families. Genera represented by two or more species were also recovered as monophyletic in the analyses (except *Tetranchyroderma* Remane, 1926). With regard to the new species from Jamaica, the analyses were unable to recover a steady position/alliance along the phylogenetic trees for it; moreover, there was no statistically significant support for the position of the new taxon at any single node (Figs 16–18).

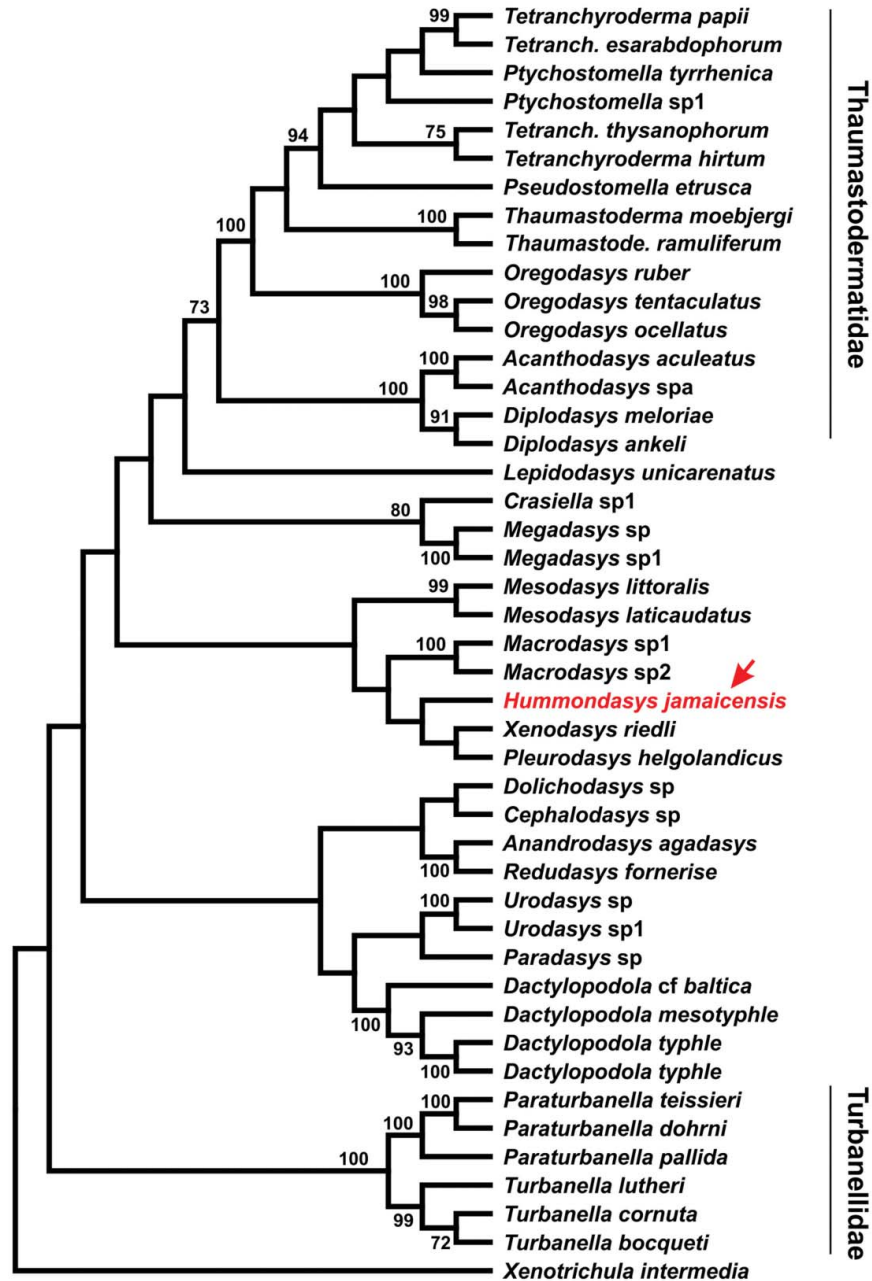
## Discussion

### Notes on diagnostic features and morphology

The total length of the studied specimens exceeding 1 mm, the very elongated and narrow body shape, along with the cuticular covering made up of only smooth cuticle (i.e. absence of spines and/or scales) and the adhesive tubes of the anterior series (TbA) originating singly and directly from the body surface, make the Jamaican gastrotrichs most similar to members of the genera *Dolichodasys* Gagne, 1977 (Fam. Cephalodasyidae), *Megadasys* (Fam. Planodasyidae) and to some extent to *Mesodasys* Remane, 1951 (Fam. Cephalodasyidae). In evident contrast with members belonging to these taxa, all of which bear a posterior body region that is rounded (unilobate), the specimens from Jamaica display a posterior body region that is shaped in the form of two caudal pedicles. This characteristic

alone not only warrants their status as a new species but also calls for the creation of a new genus to avoid a gross widening of the taxonomic boundaries of any of the above-mentioned genera. Naturally, there are additional differences in the external morphology between the gastrotrichs from Jamaica and members of the previously mentioned taxa that further justify the erection of a new genus. These differences pertain, notably, to the arrangement and number of the adhesive apparatus with special regard to the tubes of the anterior and lateral series. For example, species of *Dolichodasys* have a single TbA per side and bear lateral adhesive papillae rather than lateral adhesive tubes, while species of *Megadasys* and *Mesodasys* bears numerous TbVL/TbL/TbDL and many more TbAs in general. However, it is the peculiar layout of the reproductive system of the Jamaican worms that clearly separates them from any species of the genera *Dolichodasys*, *Mesodasys* or *Megadasys* and also from all other gastrotrich described to date. One of the key features of the Jamaican species is the direct connection of the sperm ducts with the caudal organ (copulatory organ). Among Macrodasysida, only species of a few genera bear an anatomical and functional connection between these two structures. Taxa that share this characteristic include *Mesodasys* and genera of the Thaumastodermatinae and Diplodasyinae (Fam. Thaumastodermatidae). However, in contrast with the new species from Jamaica, species of *Mesodasys* lack a frontal organ and the sperm transfer occurs by hypodermic impregnation (Ruppert, 1991; Fregni *et al.*, 1999), while members of the Thaumastodermatinae possess a single testis and the frontal and the caudal organs are attached to each other and show a luminal continuity (e.g. Ruppert, 1978; Chang *et al.*, 2002; Todaro, 2012, 2013b). Species of the two Diplodasyinae genera, *Diplodasys* Remane, 1927 and *Acanthodasys* Remane, 1927, possess reproductive systems that appear to be the most similar to that of the Jamaican species as they all include: (1) paired testes, (2) the presence of both the frontal and the caudal organs and (3) frontal and caudal organs that are anatomically separated. However, in Diplodasyinae species, the frontal organ is located anterior to the most mature egg and not posterior to it as occurs in animals from Jamaica (e.g. Ruppert, 1978; Kisielowski, 1987; Kieneke *et al.*, 2013a versus this study).

The presence in the holotype of two eggs increasing in size from posterior to anterior (see Fig. 1) indicates that in this species, oogenesis develops frontally with the precocious stages to be positioned posterior to the two visible eggs. Observations on the third adult specimen (see above) corroborate this observation and reveal furthermore that the ovary is paired and confined quite anterior along the trunk region i.e. the caudal limit of each ovary lies in the posterior vicinity of the visible eggs and anterior to the testes. This condition has several important implications. First, it helps to better differentiate the Jamaican specimens from species of the Diplodasyinae

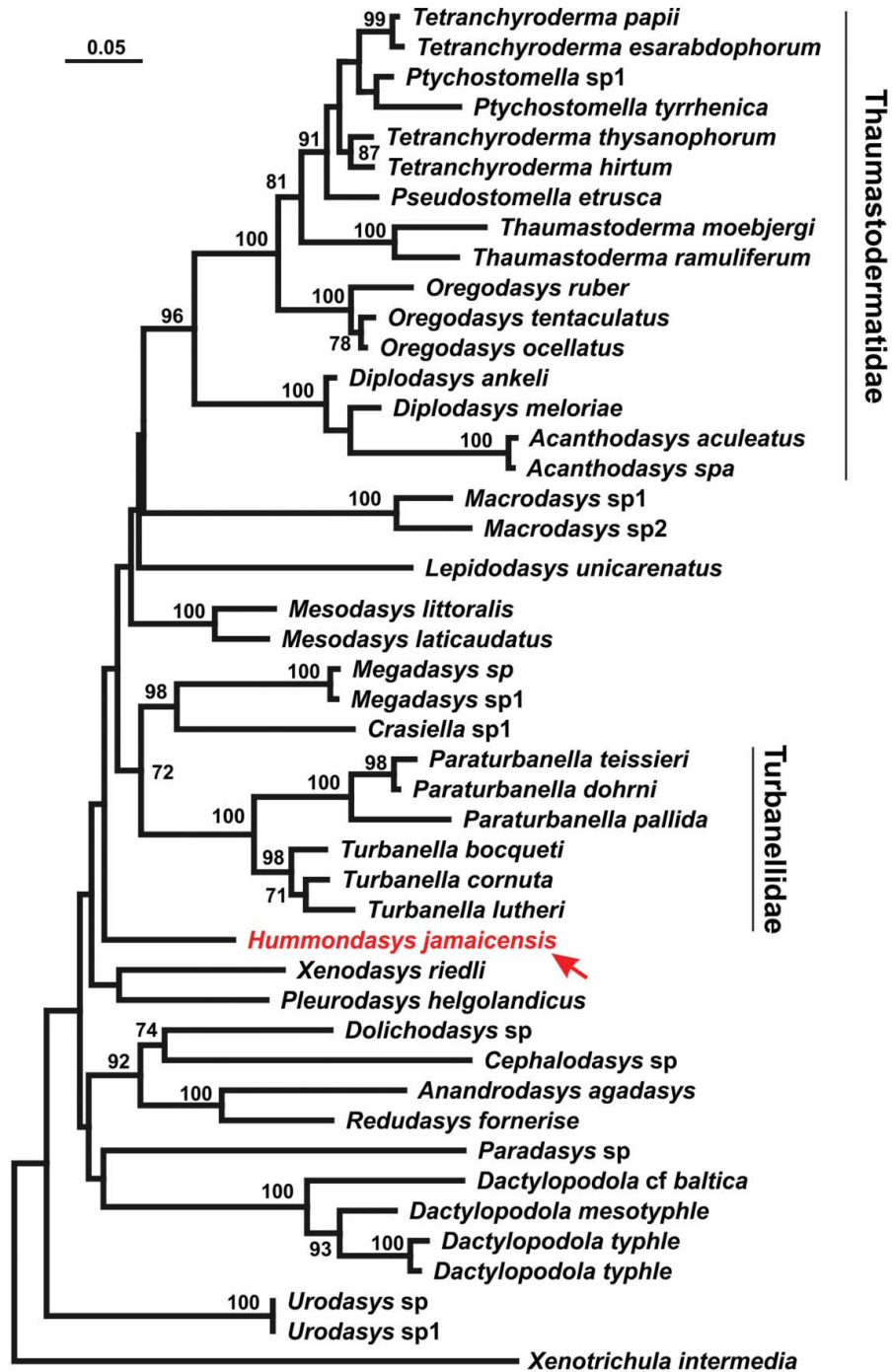


**Fig. 16.** Phylogenetic relationships of 44 Gastrotricha Macrodasysida including *Hummondasys jamaicensis* gen. et sp. nov. inferred from Maximum parsimony analysis of 18S rRNA. The outgroup is represented by *Xenotrichula intermedia* (Chaetonotida, Xenotrichulidae). Tree no. 1 out of three most parsimonious trees (length = 3854) is shown. The consistency index is (0.367120), the retention index is (0.586388) and the composite index is 0.239547 (0.215275) for all sites and parsimony-informative sites (in parentheses). Number at nodes represents bootstrap values (1000 replicates).

because in the former, the entire female gonads would be anterior to the frontal organ whereas in the latter, the single ovary is posterior to the frontal organ (Ruppert, 1978). Second, and most important from an evolutionary stand, it makes the Jamaican specimens unique, as they appear to be the only known gastrotrichs in which the female gonads are positioned anterior to the male gonads (Fig. 13).

In conclusion, traits of the external morphology along with the peculiar arrangement of the reproductive system call for both the erection of a new species and a new genus for the specimens from Negril; consequently, the name *Hummondasys jamaicensis* gen. et sp. nov. is proposed for the new taxon.

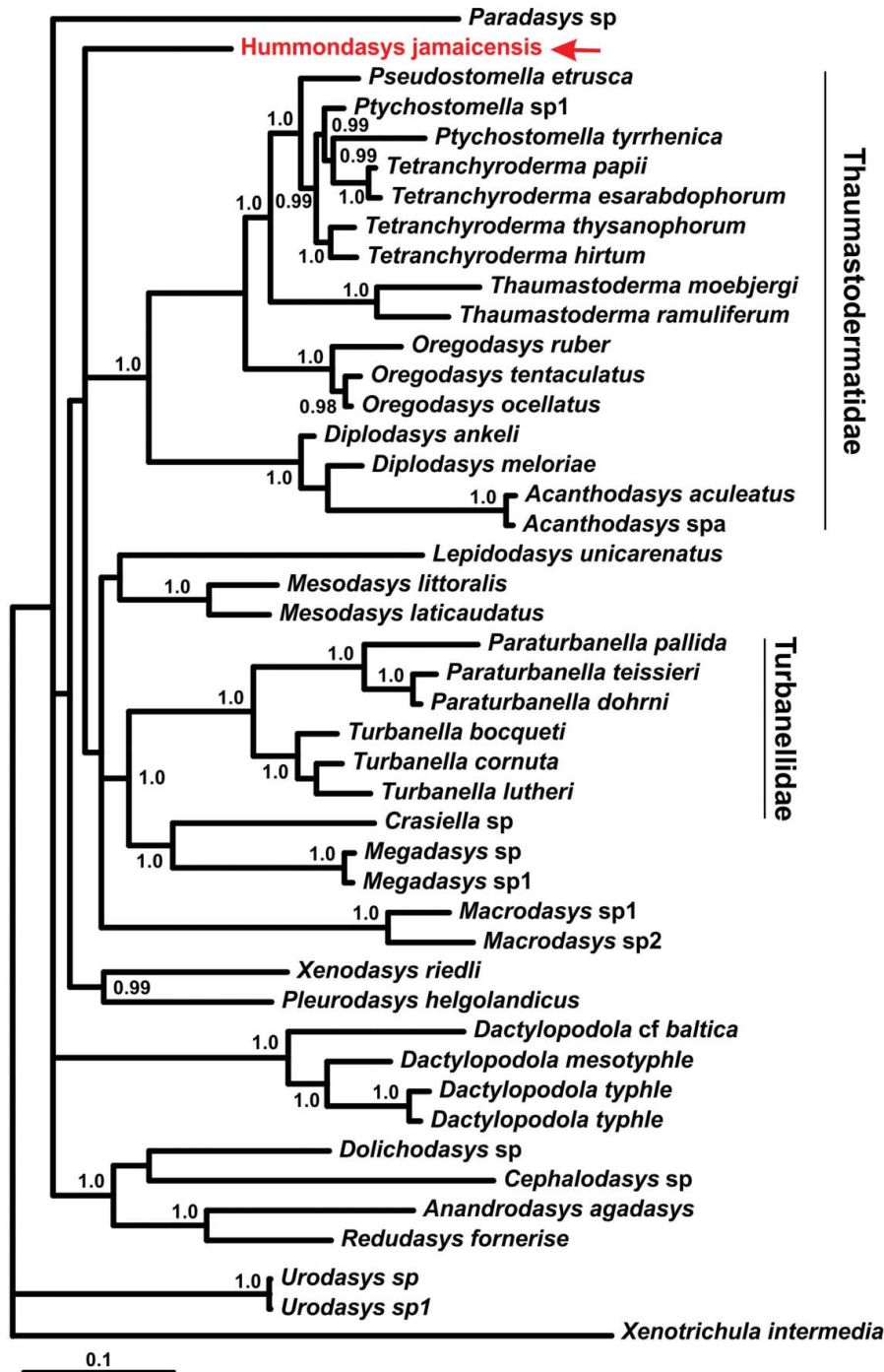
Unfortunately, the morpho-functional traits of *H. jamaicensis* gen. et sp. nov. do not permit the formulation of a



**Fig. 17.** Phylogenetic relationships of 44 Gastrotricha Macrodasysida including *Hummondasys jamaicensis* gen. et sp. nov. inferred from Maximum likelihood analysis of 18S rRNA. The outgroup is represented by *Xenotrichula intermedia* (Chaetonotida, Xenotrichulidae). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Number at nodes represents bootstrap values (1000 replicates).

preferred hypothesis concerning the affiliation of the new genus to any of the currently recognized macrodasysidan families (Todaro *et al.*, 2006a; Hummon & Todaro, 2010; Todaro *et al.*, 2012a, Guidi *et al.*, 2014). For instance,

while the caudal pedicles and the reproductive system could align *H. jamaicensis* gen. et sp. nov. to some Thaumastodermatidae, the smooth cuticle and some traits of the reproductive system make the new genus close to some



**Fig. 18.** Phylogenetic relationships of 44 Gastrotricha Macrodasysida including *Hummondasys jamaicensis* gen. et sp. nov. inferred from Bayesian analysis of 18S rRNA. The outgroup is represented by *Xenotrichula intermedia* (Chaetonotida, Xenotrichulidae). Number at nodes represents posterior probabilities.

members of the Cephalodasyidae. On the other hand, it cannot be excluded that characteristics present in *H. jamaicensis* gen. et sp. nov. and apparently shared by the other taxa are not homologous and consequently misleading in a process of natural systematization. Perhaps the phylogenetic alliance of *H. jamaicensis* gen. et sp. nov. lies

elsewhere along the Macrodasysida evolutionary tree. However, the autapomorphic characteristic of possessing separated gonads with ovaries anterior to the testes clearly distinguishes the new species from any other known gastrotrich taxon and therefore could substantiate the need for the creation of a new family (more below).

## Phylogenetic remarks

In previous studies, phylogenetic analysis based on molecular traits (18rDNA gene alone or in conjunction with the 28rDNA and Cox 1 genes) have provided support for some of the traditional grouping based on morphological traits (e.g. most genera, the families Turbanellidae and Thaumastodermatidae etc.) but have also unveiled unsuspected alliances, e.g. between species belonging to different families or between species that are apparently very different morphologically (e.g. Todaro *et al.*, 2006c, 2011b, 2012a). Some of the novelties that emerged from molecular data analysis have later been confirmed and considered to be very likely by re-examining in an evolutionary perspective the morphological characteristics of the taxa involved. This has permitted a re-systematization of some taxa thereby reducing major phylogenetic conflicts and indicating, at the same time, that in the case of contrasting phylogenetic hypotheses, those based on molecular markers are the most robust. This view is best supported by the recent cases of *Anadrodasys agadasys*, formerly described as *Dactylopodola agadasys* in the family Dactylopodolidae and now reunited with *Redudasys fornerise* in the family Redudasyidae (see Todaro *et al.*, 2012a), and *Megadasys*, previously affiliated with the family Cephalodasyidae, but recently united with *Cra-siella* in the family Planodasyidae (see Guidi *et al.*, 2014).

Based on these results, it was hoped that a molecular analysis based on the 18rRNA gene and involving all of the relevant genera and families of the Macro-dasyida could provide support to the alliance of *H. jamaicensis* gen. et sp. nov. with known gastrotrich taxa. Our best supposition was that the molecular analysis would substantiate the alliance of the new taxon with either *Mesodasys* (within Cephalodasyidae) or Diplodasyinae (within Thaumastodermatidae) as these gastrotrichs share potential morphological synapomorphies with the new species (see above). An alternative possibility was that the analysis would unveil a close relationship between the new genus and other less-similar taxa, consequently paving the way for studies looking for morphological synapomorphies that are so far undetected. Unfortunately, in this regard, neither hypothesis was verified. In short, both the unstable position in the phylogenetic trees and the low statistical support at the respective nodes make any attempt to affiliate *H. jamaicensis* gen. et sp. nov. with any of the currently recognized families as extremely speculative.

## Conclusions

The uncertainties derived from the comparisons of the morphological traits and the failure of the phylogenetic analysis to find an alliance of the specimen from Jamaica with the involved taxa suggest the erection of a new family to

affiliate *H. jamaicensis* gen. et sp. nov.; consequently, the establishment of Hummondasyidae fam. nov. is proposed.

The difficulties faced by the molecular analysis, based on the 18S rRNA gene, to find a close alliance of *H. jamaicensis* gen. et sp. nov. among the known gastrotrich taxa, a condition shared with *Lepidodasys*, may be interpreted as an early divergence of these gastrotrichs along the Macro-dasyida evolutionary branch. It is worth mentioning that, within Gastrotricha, deep divergences appear unsolved, as in previous analyses based on multiple genes (e.g. Todaro *et al.*, 2011b; Kanneby *et al.*, 2013) and likely necessitate a metagenomics approach.

Our knowledge of the biodiversity and phylogeny of the Gastrotricha are far from complete; however, Todaro in Appeltans *et al.* (2012) estimated the number of morphological species yet to be discovered to range from 1310 to 1810. The finding of *H. jamaicensis* gen. et sp. nov. suggests that among those, there may be some highly significant species from a phylogenetic point of view, the anatomical peculiarities of which may shed light on the evolution of these creatures and consequently helpful for the process of their natural systematization.

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