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Genus *Baseodiscus* (Nemertea: Heteronemertea): Molecular identification of a new species in a phylogenetic context

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Abstract

A new heteronemertean, *Baseodiscus jonasii* sp. nov., is described from Guadalcanal, Solomon Islands. It resembles *B. delineatus* in inner morphology but can be distinguished from this species by its different colour pattern and differences in the nucleotide sequence of the mitochondrial 16S rRNA gene. The monophyletic status of the genus is investigated by reconstructing the phylogeny of six specimens from four species assigned to this genus, together with 22 specimens from nine other heteronemertean genera, using parsimony and Bayesian analysis. The results imply that *Baseodiscus* is a monophyletic group while several other heteronemertean genera are non-monophyletic.

Keywords: 16S rRNA, *Baseodiscus*, *Baseodiscus jonasii* sp. nov., *Heteronemertea*, mitochondrial DNA, Solomon Islands

Introduction

The genus *Baseodiscus* (Diesing, 1850) comprises about 35 described species (Gibson 1995), most of which are tropical or subtropical. Species assigned to this genus consist of large, marine, benthic worms with a weakly developed proboscis and no horizontal cephalic slits (Gibson 1979). One species of *Baseodiscus*, *B. quinquelineatus* (Quoy and Gaimard, 1833), has previously been reported from the Solomon Islands. This species has been redescribed in detail by Gibson (1979), together with *B. delineatus* (Delle Chiaje, 1825), which is the type-species for the genus *Baseodiscus* (Gibson 1995). In spite of his thorough investigation, Gibson (1979) found only minor differences in internal morphology between the two species and concluded that the most distinctive character for *B. quinquelineatus* is the colour pattern. The new taxon described here, *Baseodiscus jonasii* sp. nov., is morphologically very similar to the two species mentioned but has a distinguishable colour pattern. Four specimens of the new species were examined but could not be distinguished

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Table I. These characters were checked through histological investigation. In comparison with corresponding characters of *Baseodiscus delineatus* we found no divergence.

External features ^a	Body wall and musculature	Proboscis apparatus	Nervous system and cerebral organs	Cephalic glands and sense organs	Alimentary canal	Blood system
Length	Organization	Length	Cerebral ganglia	Frontal organ	Placement of	Cephalic blood
Width	of body wall	Layers	Commissures	Eyes	mouth	supply
Colour	Dermis	Nerves	Foregut nerves	Type of eyes	Gut division	Lacunar
Head	Epidermis	Rhynchodaeum	Cerebral organs	Distribution	Foregut	arrangement
Eyes		epithelium	Lateral nerve	of glands	epithelium	Placement of
Mouth		Rhynchocoelic	cords		Foregut wall	blood vessels
Proboscis pore		villus			Intestinal wall	
Cephalic slits		Rhyncocoel			Intestinal	
		endothelium			epithelium	

^aExternal features: length about 60 cm, width about 4 mm, ground colour reddish brown with numerous longitudinal yellowish beige stripes extending full length of body. Head rounded, clearly demarcated from trunk (Figure 3). Eyes not clearly visible in living specimens but can be seen on histological sections, lying beneath epidermis. Mouth situated ventrally, just behind the cerebral ganglia. Proboscis pore subterminal. Cephalic slits absent. Head retracted into trunk upon preservation. Excretory system: it has not been possible to observe any details of the excretory system. Reproductive system: gonads not present in sections examined.

from *B. delineatus* when comparing the internal morphological characters listed in Table I. *Baseodiscus jonasii* sp. nov. is, however, genetically separated both from specimens of *B. delineatus* (from Rottneest Island, Australia and from Ischia, Italy), and from a specimen of *B. quinquelineatus* from the Solomon Islands which supports our view that this is a species new to science.

We also used the 16S rRNA sequences from 28 heteronemertean and three hoplonemertean specimens listed in Table II to test whether the genus *Baseodiscus* is monophyletic, using Bayesian and parsimony analyses. The results indicate, even though we have only six specimens from four species represented, that *Baseodiscus* is a monophyletic group. Other heteronemertean genera included in analyses are non-monophyletic. This result is in concordance with earlier studies (Sundberg and Saur 1998; Thollessen and Norenburg 2003).

Material and methods

Specimens

The specimens of the new taxon were all collected intertidally at low tide, among dead corals on reef flats, from three localities along the northern coast of Guadalcanal, Solomon Islands (Rove near White River, west of Honiara, Mendana Reef and Vulelua Island) in June 1995. Their external features were examined after anaesthetizing with MgCl₂. The morphological description is based on histological examination of four specimens (one from Rove and three from Mendana Reef) preserved in 40% formalin, embedded in 56°C m.p. paraffin wax, sectioned at 7 µm and stained with the Mallory trichrome method. For DNA extraction specimens were placed in 70% ethanol. The type

Table II. List of nemertean species included in analysis with localities and accession numbers.

Species	Locality	Accession no.
Heteronemertea		
<i>Baseodiscus jonasii</i> sp. nov.	Guadalcanal, Solomon Islands	AY955230
	Guadalcanal, Solomon Islands	AY955231
<i>Baseodiscus delineatus</i> (Delle Chiaje, 1825)	Rottneest Island, Australia	AY955232
<i>Baseodiscus delineatus</i>	Ischia, Italy	AY955227
<i>Baseodiscus quinquelineatus</i> (Quoy and Gaimard, 1833)	Solomon Islands	AY955228
<i>Baseodiscus hemprichii</i> (Ehrenberg, 1831)	Hurghada, Egypt	AY955229
<i>Lineus ruber</i> (Müller, 1774)	Sweden	AF103758 ^a
	Sweden	AF103759 ^a
	UK	AF103757 ^a
<i>Lineus viridis</i> (Müller, 1774)	UK	AF103760 ^a
	Sweden	AF103760 ^a
	Manset, ME, USA	AJ436826 ^b
<i>Lineus longissimus</i> (Gunnerus, 1770)	Plymouth area, UK	AF103763 ^a
	Anglesey, UK	AJ436825 ^b
<i>Lineus albostratus</i> Takakura, 1898	Vostok Bay, Sea of Japan, Russia	AJ436822 ^b
<i>Tenuilineus bicolor</i> (Verrill, 1892)	Sebastian Inlet, FL, USA	AJ436823 ^b
<i>Notospermus geniculatus</i> (Delle Chiaje, 1828)	Seto, Japan	AJ436824 ^b
<i>Oxypolella alba</i> Bergendal, 1903	Tjärnö, Sweden	AF103767 ^a
<i>Micrura purpurea</i> (Dalyell, 1853)	Tjärnö, Sweden	AF103766 ^a
<i>Micrura fasciolata</i> Ehrenberg, 1828	Tjärnö, Sweden	AF103765 ^a
<i>Micrura alaskensis</i> Coe, 1901	San Juan Island, WA, USA	AJ436827 ^b
<i>Ramphogordius sanguineus</i> (Rathke, 1799)	Wales, UK	AF103761 ^a
	Anglesey, UK	AJ436828 ^b
<i>Cerebratulus marginatus</i> Renier, 1804	Washington State, USA	AJ436821 ^b
<i>Cerebratulus</i> sp.	Tjärnö, Sweden	AF103755 ^a
<i>Riseriellus occultus</i> Rogers, Junoy, Gibson and Thorpe, 1993	Wales, UK	AF103764 ^a
<i>Parvicirrus dubius</i> (Verrill, 1879)	Georgetown, ME, USA	AJ436830 ^b
<i>Parborlasia corrugatus</i> (McIntosh, 1876)	McMurdo Sound, Antarctica	AJ436829 ^b
Hoploneurtemea (outgroup)		
<i>Amphiporus angulatus</i> (Müller, 1774)	Cobscook, ME, USA	AJ436786 ^b
<i>Amphiporus formidabilis</i> Griffin, 1898	San Juan Island, WA, USA	AJ436787 ^b
<i>Tetrahymena elegans</i> (Girard, 1852)	Nahant, MA, USA	AJ436810 ^b

^aSundberg and Saur (1998); ^bThollessen and Norenburg (2003).

specimens of the new taxon are deposited at the Museum of Natural History (MNHG) in Göteborg, Sweden.

DNA sequencing

DNA was extracted using the QIAamp DNA Mini Kit for tissue (QIAGEN Inc.) following the protocol supplied by the manufacturer. A part of the 16S gene was amplified by polymerase chain reaction (PCR) using a thermal cycler (PTC-100 Programmable Thermal Controller, MJ Research Inc.) and the universal primers 16Sar-L and 16Sbr-H (Palumbi 1996). Each PCR was performed with 20–80 ng of DNA template in a 50 µl reaction volume (10 mM Tris-HCl, 50 mM KCl, 2 mM MgCl₂, 0.3 µM of each primer, 100 µM of each dNTP, 2 units (0.04 U/µl) of Taq DNA polymerase (Sigma Product No. D6677)). Thermal cycling started with 2 min of denaturation at 94 °C followed by 60 cycles of 30 s at 94 °C, 30 s at 47 °C and 1 min at 72 °C. The cycling was ended with a 7 min

extension phase at 72°C. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Inc.). Sequencing was carried out with Cy5-labelled primers (16Sar-L and 16Sbr-H) on an ALFexpress automated sequencer (Pharmacia) following standard procedures with primer concentration 0.09 µM in the sequencing reactions.

Outgroup

Sundberg et al. (2001) as well as Thollessen and Norenburg (2003) have shown that hoplonemerteans and heteronemerteans are both monophyletic groups. In this study we used three hoplonemertean species as outgroup.

Alignment and phylogenetic analysis

The sequences were edited and aligned with Lasergene (DNASTAR) using the Clustal-V (Higgins et al. 1992) algorithm; alignment can be obtained from the corresponding author. Gap/gap length penalties were set to 15/8. Ambiguously aligned regions were excluded using MacClade 4.0 (Maddison and Maddison 2001). PAUP 4.0b10 (Swofford 2002) was used for the maximum parsimony analysis, using a heuristic search strategy (TBR), random addition, five replicates. Clade support was assessed with non-parametric bootstrap from 1000 replicates. Phylogenetic analysis using Bayesian inference was performed with MrBayes ver. 3.06 (Huelsenbeck and Ronquist 2001) using default values of four Markov chains, with invariant sites and gamma distribution, lset nst=6 (GTR). The Monte Carlo Markov chain (MCMC) length was 1,000,000 generations with sampling of every 100th generation chain. Log-likelihood values for sampled trees stabilized after approximately 100,000 generations, burn-in was set to 5000 leaving the last 5000 sampled trees for estimating posterior probabilities (Bayesian support values). Five separate analyses were run starting from random trees to ensure congruence.

Results and discussion

After excluding ambiguous regions the aligned data set contained 486 nucleotide positions of which 284 were parsimony informative. Figure 1 shows the resulting majority rule consensus tree from the Bayesian analysis and Figure 2 shows the resulting tree of the parsimony analysis. All species in the taxon *Baseodiscus* form a monophyletic group with posterior probability 1.00 and bootstrap support 98%. Within this group, expressed as percentage nucleotide dissimilarity, the two specimens of *B. jonasii* nov. sp. diverge from *B. delineatus* with 21.1%, and from *B. quinquelineatus* with 22.0%. The divergence between *B. delineatus* and *B. quinquelineatus* is 10.1%. Both analyses indicate that the genera *Lineus*, *Micrura*, and *Cerebratulus* are non-monophyletic.

Many of the species assigned to the genus *Baseodiscus* are inadequately described (Gibson 1995). Most of the descriptions were made during the years 1825 (Delle Chiaje 1825: *B. delineatus*) to 1934 (Coe 1934: *B. edmondsoni*) and some are based on preserved animals that may have lost both shape and colours. A few have been redescribed since then: *B. antarcticus* (Gibson 1985), *B. aureus*, *B. mexicanus* (Friedrich 1970), *B. delineatus*, *B. hemprichii*, *B. quinquelineatus* (Gibson 1979), *B. lumbricoides* (Gibson and Ogren 1990), *B. unistriatus* (Gibson 1974), but most still lack a thorough description. Genetic information has never before been used in descriptions of *Baseodiscus* species. *Baseodiscus jonasii* sp. nov. has a colour pattern that is distinctive from all other *Baseodiscus* species

Figure 1. Parsimony tree based on the 16S rRNA data with bootstrap support values from 5000 replicates (heuristic search, random additions, five replicates).

Figure 2. Majority rule consensus tree for the 16S rRNA data resulting from the Bayesian analysis (model GTR+G+I), 1,000,000 generations. Numbers refer to posterior probabilities.

except *Baseodiscus delineatus*, which it resembles both externally and internally. According to Gibson (1979), the coloration of *B. delineatus* is “a uniform dull yellowish-fawn, marked by light reddish-brown longitudinal lines which extend for the full body length”. He also comments that “each stripe is of variable width and outline and adjacent stripes occasionally join with each other”. It is easy to imagine that the dark lines might have become broader in some populations of worms, rendering the animal with a reddish brown ground coloration and yellowish stripes. There also seems to be some colour variation within *B. delineatus*, especially when *B. delineatus* var. *curtus* is included in the species (some authors prefer to treat *Baseodiscus curtus* (Hubrecht 1879) as a separate species, but here we choose to follow Gibson’s suggestion (Gibson 1979, 1995) to synonymize it with *B. delineatus*). Hubrecht (1879) described *Polia curta*, later transferred to the genus *Baseodiscus* by Bürger (1904), as distinguished from *B. delineatus* in that “the brown stripes are much more closely set on the back, 12–15 being counted in a transverse line on the back”. The colour pattern of *B. delineatus* was described by Hubrecht (1879) as “dark brown stripes longitudinally intersecting the light brown ground colour ... about five to seven may be counted in a transverse line across the back”. Bürger (1904) described *B. delineatus* and *B. curtus* as even more variable in colour. He stated that the ground coloration for *B. delineatus* is light brown or olive green with dark brown longitudinal stripes, and for *B. curtus* as yellowish grey, brown, reddish brown or red (“zinnoberrot”) with brown longitudinal stripes. There has, however, never been any record of a reddish brown worm with yellowish longitudinal stripes. We could have expanded the taxon *B. delineatus* to contain just another colour variant. However, 16S mtDNA sequences from *B. jonasii* sp. nov., *B. delineatus* (type species of the genus, and which it resembles most), and *B. quinquelineatus* (the only *Baseodiscus* species previously reported from the Solomon Islands (Gibson and Sundberg 2002)) indicate a clear genetic difference between all three species. The two *B. delineatus* sequences are identical even though the specimens were collected as far apart as Australia and Italy, the two *B. jonasii* sp. nov. specimens differ only in one nucleotide. The difference between *B. delineatus* and *B. quinquelineatus* is about 10%, and *B. jonasii* sp. nov. differs from the other two by more than 20%. We therefore conclude that *B. jonasii* sp. nov. is previously undescribed and unnamed.

Nemerteans have in general few external characters and are therefore usually described using internal morphology. There are, however, problems with most internal characters since nemerteans contract during fixation and preservation. The relative position of organs and the thickness of different tissues are quite variable, and depend on the amount of body contraction. Many morphological characters that have been used in earlier descriptions are therefore not reliable. This study gives an example of a genetically well-defined taxon that is hard to distinguish when looking, as is the tradition when describing nemerteans, merely for internal morphological characters. *Baseodiscus jonasii* sp. nov. has a characteristic colour pattern, different from that of *B. delineatus*, but the two taxa are morphologically indistinguishable. Without the genetic information the new species would not have been identified. This result is probably not a rare case. Presumably, if we were to look into several nemertean species, we would find many more species in some cases, and in other cases the opposite since some species for certain have a range of variation in pigmentation and pattern within the species, e.g. *Oerstedia dorsalis* (Sundberg and Janson 1988). With an increasing knowledge of genetics we conclude that speciation quite commonly takes place without an apparent morphological diversification visible to us (Strand and Sundberg 2005), and that, at least in some cases, we need a new approach both for describing nemerteans and for erecting new species, genera, and families.

Baseodiscus jonasii sp. nov.
(Figure 3; Table I)

Type material

Holotype: MNHG catalogue number 78, of unknown sex, transverse sections of the anterior end, 18 slides (marked SOL6/1). The 16S rRNA gene sequence of the holotype is deposited in Genbank (accession number AY955230). Paratypes: three (marked SOL6/2 (39 slides), SOL6/3 (13 slides), SOL6/4 (20 slides)) of unknown sex, transverse sections of the anterior end and in one specimen (SOL6/2) various mid-body regions.

Type locality

Mendana Reef, Honiara, Solomon Islands. Intertidal, among dead corals on reef flats.

Etymology

The name is dedicated to the finder of the first specimen, Jonas Sundberg.

External features

Length about 60 cm, width about 4 mm, ground colour reddish brown with numerous longitudinal yellowish beige stripes extending full length of body. Head rounded, clearly demarcated from trunk (Figure 3). Eyes not clearly visible in living specimens but can be seen on histological sections, lying beneath epidermis. Mouth situated ventrally, just behind the cerebral ganglia. Proboscis pore subterminal. Cephalic slits absent. Head retracted into trunk upon preservation.

Internal morphology

Indistinguishable from *Baseodiscus delineatus* (Table I).

Figure 3. External view of *Baseodiscus jonasii* sp. nov. Drawing made by Ray Gibson.

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