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Authors: Abato, Jamael, Along, Archie, Aspe, Nonillon, Awa, Caryl Kaye, Burias, Nick Anthony, et al.

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Morphology and Phylogeny of *Notospermus psittacinus* comb. nov. (Nemertea: Pilidiophora: Lineidae) From Mindanao, Philippines

Jamael Abato^{1,2*}, Archie Along³, Nonillon Aspe⁴, Caryl Kaye Awa⁵,
Nick Anthony Burias³, Laurence Calagui³, Shirlamaine Irina Calagui³,
Meljan Demetillo³, April Joy Jovita³, Julius Anthony Leones³,
Allexc Mae Niez⁵, Romell Seronay³, and Hiroshi Kajihara²

¹Biology Department, College of Natural Sciences and Mathematics, Mindanao State University–Main Campus, Marawi City 9700, Lanao del Sur, Philippines

²Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan

³Department of Biology, College of Mathematics and Natural Sciences, Caraga State University, Butuan City 8600, Agusan del Norte, Philippines

⁴Department of Environmental Science, College of Environment and Life Sciences, Mindanao State University at Naawan, Naawan 9023, Misamis Oriental, Philippines

⁵Department of Marine Biology, College of Marine and Allied Sciences, Mindanao State University at Naawan, Naawan 9023, Misamis Oriental, Philippines

This study provides a redescription of the lineid heteronemertean *Notospermus psittacinus* (Bürger, 1890) comb. nov. based on specimens collected from Mindanao, Philippines. The species was previously assigned to the genus *Lineus* Sowerby, 1805, and has not been reported since its original description from Ambon, Indonesia. No DNA barcode sequences based on reliably identified material from the type locality are currently available. *Notospermus psittacinus* comb. nov. differs from the other six named species of the genus in body coloration, but can be distinguished from *N. annulatus* (Grube, 1840) only by molecular and biogeographic evidence. Despite the observed morphological variations in our specimens, genetic distance analyses indicated that all represent a single species (cytochrome *c* oxidase subunit I [COI] *p*-distance = 0%–2.12% [*n* = 7], 16S ribosomal RNA [16S] *p*-distance = 0%–1.57% [*n* = 6]). Genetic divergence between *N. annulatus* and *N. psittacinus* comb. nov. was 11.39%–12.18% for the 16S gene and 5.79% for the histone H3 (H3) gene, supporting their status as distinct species. A multigene phylogenetic analysis based on 16S, 18S ribosomal RNA, 28S ribosomal RNA, COI, and H3 sequences showed that all *N. psittacinus* comb. nov. specimens formed a well-supported clade within the genus *Notospermus* Huschke, 1830. Although intra-generic relationships remain poorly resolved, this result corroborates the generic reassignment of the species from *Lineus* to *Notospermus*.

Key words: biodiversity, cryptic species, DNA barcoding, Heteronemertea, STMIP

INTRODUCTION

Notospermus Huschke, 1830 is a small lineid genus currently comprising six described species worldwide (Norenburg et al., 2025), all of which can be distinguished by external coloration and pigmentation patterns. A recent taxonomic revision by Kajihara et al. (2022), integrating body coloration and phylogenetic data, clarified the taxonomic identities of *Notospermus annulatus* (Grube, 1840), *Notospermus*

fuscoviridis (Takakura, 1898), *Notospermus geniculatus* (Delle Chiaje, 1822), and *Notospermus mitellatus* (Takakura, 1898). Among the described congeners, the greenish *N. annulatus* and *N. geniculatus* are particularly similar in morphology and were once regarded as synonymous (Bürger, 1904). As noted by Kajihara et al. (2022), *N. annulatus* exhibits dorsally discontinuous white rings, whereas these rings are continuous in *N. geniculatus*. In addition to these morphological traits, the two species—originally described from the Mediterranean Sea—also differ substantially in their 16S rRNA gene sequences and form distinct phylogenetic lineages within the genus.

* Corresponding author. E-mail: jamaelabato@gmail.com
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The nominal lineid heteronemertean *Cerebratulus psittacinus* Bürger, 1890 was originally described from Ambon, Indonesia (Bürger, 1890) and later transferred to the genus *Lineus* Sowerby, 1805 by Bürger (1895: 620). The original illustration of its external appearance was based on a fixed specimen and, therefore, is not highly informative. Nevertheless, it shows that the anterior-most white ring projects forward mid-dorsally, as observed in *N. annulatus*, *N. geniculatus*, and *N. mitellatus*. This resemblance suggested a possible affinity of *Lineus psittacinus* with *Notospermus* (Kajihara et al., 2016). However, the species has not been reported since its original description.

Research on ribbon-worm diversity in the Philippines is in its infancy, and recent surveys indicate that *Notospermus* has not yet been documented in the country (Abato et al., 2025). However, during an intertidal ribbon-worm faunal survey conducted in Mindanao in 2024, greenish *Notospermus* specimens resembling *N. annulatus* and *L. psittacinus* were collected. In this study, we apply both morphological and molecular approaches to determine the identity of these specimens. Molecular species delimitation was performed using the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA (16S) to assess how many putative species were represented among the collected specimens and whether they are different from the morphologically similar *N. annulatus*. In addition, a multigene phylogenetic analysis using 16S ribosomal RNA (16S), 18S ribosomal RNA (18S), 28S ribosomal RNA (28S), COI, and histone H3 (H3) genes was conducted to evaluate the phylogenetic placement of the Philippine species within *Notospermus*.

MATERIALS AND METHODS

Sample collection, documentation, and processing

Prior to specimen collection, a Gratuitous Permit (GP No. 0253-23) was obtained from the Bureau of Fisheries and Aquatic Resources, Department of Environment and Natural Resources. A total of 10 *Notospermus* specimens were collected in 2024 under the Systematics and Taxonomy of Marine Invertebrates from the Philippines (STMIP) project: *i*) five specimens from Brgy. Poblacion, Naawan, Misamis Oriental; *ii*) two specimens from Brgy. Pagahan, Initao, Misamis Oriental; *iii*) one specimen from Brgy. Vinapor, Carmen, Agusan del Norte; and *iv*) two specimens from Brgy. Poblacion, Lianga, Surigao del Sur. All specimens were collected from intertidal rocks and processed following the methods of Abato et al. (2025).

Voucher materials have been deposited in the Philippine Zoological Reference Collection at the National Museum of Natural History, National Museum of the Philippines, Rizal Park, Manila, Philippines (NMA). The nucleotide sequences generated in this study have been deposited in GenBank.

DNA extraction, PCR amplification, and sequencing

DNA extraction, PCR amplification of the COI, and sequencing followed the protocols of Abato et al. (2022), except that gene markers were sequenced using QuantumDye Terminator v3.1 (QuantumSeq, USA). When the standard PCR conditions did not yield results, a touch-down PCR protocol was applied: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 45°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 35°C, 40 sec at 72°C), followed by 7 min at 72°C. The PCR conditions for the amplification of the 16S, 18S, 28S, and H3, as well as sequencing and base calling procedures, also followed Abato et al. (2022). To verify sequence accuracy and assess potential contamination, MegaBLAST searches (Morgulis et al., 2008) were performed through the NCBI database (<http://www.ncbi.nlm.nih.gov/>).

These searches were also used for preliminary species- and higher-taxon-level identifications of the collected specimens.

Molecular species delimitation, genetic distance, and phylogenetic analyses

Partial sequences of the 16S, 18S, 28S, COI, and H3 genes from Lineage E and Lineage F of the family Lineidae (Kajihara et al., 2022), along with other available sequences of *Notospermus* spp., *Cerebratulus albifrons* Coe, 1901, and *Quasimicrura dellechiaiei* (Hubrecht, 1879), were downloaded from GenBank (Table 1) and combined with sequences generated in the present study for molecular analyses. '*Notospermus*' spp. IZ 132528 and IZ 134234 of Kvist et al. (2014) from Panama and Australia were not included in the analyses, as they are not true congeners; the former could represent *Corsoua kristenseni* Corrêa, 1963 (Hookabe and Kajihara, 2020).

Molecular species delimitation analyses based on COI and 16S sequences of the Philippine specimens and *Notospermus* spp. including *N. annulatus* were conducted using three methods: Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021), and Poisson Tree Processes (PTP) (Zhang et al., 2013) accessed and downloaded from [itaxotools](https://itaxotools.org/) (<https://itaxotools.org/>). All analyses were executed in default settings. ASAP and ABGD analyses were based on aligned COI (615 bp) and 16S (519 bp) sequences, whereas the bPTP analysis used an input tree generated in IQ-TREE ver. 2.1.3 (Minh et al., 2020) using the same gene markers. Uncorrected genetic distances for 16S, COI, and H3 were calculated in MEGA ver. 12.0.11 (Kumar et al., 2024) using default parameters.

For the maximum likelihood (ML) and Bayesian inference (BI) analyses, COI and H3 datasets were aligned first in MEGA ver. 12.0.11 using the MUSCLE algorithm (Edgar, 2004), whereas 16S, 18S, and 28S datasets were aligned in MAFFT ver. 7 online version (Katoh et al., 2019). Ambiguously aligned sites were trimmed using ClipKIT ver. 1.3.0 (Steenwyk et al., 2020), resulting in datasets of 527 bp (16S), 1772 bp (18S), 2842 bp (28S), 615 bp (COI), and 331 bp (H3), which were concatenated in MEGA ver. 12.0.11.

The best-fit partitions and substitution models for ML and BI were determined using PartitionFinder ver. 2.1.1 (Lanfear et al., 2017) employing the greedy algorithm (Lanfear et al., 2012), yielding seven subsets and corresponding models: subset 1, COI (first codon), TRNEF + G; subset 2, COI (second codon), 18S, H3 (second codon), K80 + G; subset 3, COI (third codon), HKY + G; subset 4, H3 (first codon), K80 + G; subset 5, H3 (third codon), JC; subset 6, 16S, HKY + I + G; and subset 7, 28S, TRN + G. ML analyses were executed using IQ-TREE ver. 2.1.3 applying the suggested models to each subset, with branch support values derived from 1000 ultrafast bootstrap (UFBoot) (Minh et al., 2013), and RAxML ver. 8.2.10 (Stamatakis, 2015) using the GTR + G model to all subsets with branch support values calculated from 1000 bootstrap pseudoreplicates (BS). BI was performed using MrBayes ver. 3.2.6 x64 (parallel version) (Ronquist and Huelsenbeck, 2003; Altekar et al., 2004) employing models GTR + G for subsets 1 and 7; K2P for subsets 2 and 4; HKY + G for subset 3; JC for subset 5; and HKY + I + G for subset 6, launching two parallel runs with four Markov chains in each run (three cold and one hot) for 5×10^7 generations sampling every 100 generations from the chain. Run convergence was assessed using the standard deviation of split frequencies (0.001825), estimated sample sizes for all parameters (> 6281), and potential scale reduction factors for all parameters (1.000).

RESULTS AND DISCUSSION

Notospermus psittacinus (Bürger, 1890) (Fig. 1A–Q)

Cerebratulus psittacinus Bürger, 1890: 13–14, pl. I, fig.

Table 1. List of species included in the phylogenetic analyses, GenBank accession numbers for 16S, 18S, and 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), histone H3 (H3) gene markers, and their references.

Species	Catalogue number or isolate	GenBank accession numbers					Reference
		16S	18S	28S	COI	H3	
<i>Cerebratulus albifrons</i>		KU197368	—	—	KU197713	—	Hiebert 2016
<i>Notospermus albocinctus</i>	Co_Not_002	PQ100261	—	—	PQ100336	—	Ellison et al. unpublished
<i>Notospermus albocinctus</i>	Pili_CB064_1503_081111_1	MW022088	—	—	MW021907	—	Maslakova et al. unpublished
<i>Notospermus annulatus</i>	MCZ:IZ1:32741	KF935462	KF935295	KF935351	—	KF935407	Kvist et al. 2014
<i>Notospermus cf. tricuspidus</i>	BOMAN_02062	—	—	—	PP834831	—	Maslakova et al. 2025
<i>Notospermus fuscoviridis</i>	ICHUM 6202	LC625659	LC625684	LC625692	—	LC625716	Kajihara et al. 2022
<i>Notospermus geniculatus</i>	ICHUM 6204	LC625661	—	LC625694	—	LC625718	Kajihara et al. 2022
<i>Notospermus geniculatus</i>		MH714705	—	—	MH714705	—	Jiang and Deng 2018
<i>Notospermus mitellatus</i>	ICHUM 6203	LC625660	LC625685	LC625693	LC625629	LC625717	Kajihara et al. 2022
<i>Notospermus psittacinus</i> comb. nov.	NMA 4721	PX563043	—	—	PX508211	PX511570	present study
<i>Notospermus psittacinus</i> comb. nov.	NMA 4722	—	—	PX563046	PX508215	PX511571	present study
<i>Notospermus psittacinus</i> comb. nov.	NMA 4723	PX563001	PX563049	PX563047	PX508212	PX511572	present study
<i>Notospermus psittacinus</i> comb. nov.	NMA 4724	PX563030	—	—	PX508213	PX511573	present study
<i>Notospermus psittacinus</i> comb. nov.	NMA 4725	PX563002	—	PX563048	PX508214	PX511574	present study
<i>Notospermus psittacinus</i> comb. nov.	NMA 4726	PX563037	PX563050	—	PX508217	PX511575	present study
<i>Notospermus psittacinus</i> comb. nov.	NMA 4727	PX563000	—	—	PX508216	PX511576	present study
<i>Notospermus</i> sp.	MCZ:IZ:133726	KF935464	—	—	—	KF935409	Kvist et al. 2014
<i>Notospermus</i> sp.	MCZ:IZ:135356	KF935466	KF935299	KF935355	—	KF935411	Kvist et al. 2014
<i>Notospermus</i> sp.	MCZ:IZ:132528	KF935463	KF935296	KF935352	KF935515	KF935408	Kvist et al. 2014
<i>Notospermus</i> sp.	MCZ:IZ:134234	KF935465	KF935298	KF935354	KF935516	KF935410	Kvist et al. 2014
<i>Notospermus</i> sp.	ETP033_B1_67	PP865521	—	—	—	—	Ellison et al. unpublished
<i>Notospermus</i> sp.	Pili_ETP033_SMPP0009				PP865359		Ellison et al. unpublished
<i>Notospermus</i> sp.	BOMAN_09092	—	—	—	PP834848	—	Maslakova et al. 2025
<i>Notospermus</i> sp.	BOMAN_09061	—	—	—	PP834846	—	Maslakova et al. 2025
<i>Quasimicrura dellechiaiei</i>		KF935461	KF935294	KF935350	KF935514	KF935406	Kvist et al. 2014
<i>Riserius</i> sp. “eyes”		KC777029	—	KU365719	KC777038	—	Hiebert et al. 2013
<i>Riserius</i> sp. “no eyes”		KC777026	—	—	KC777034	—	Hiebert et al. 2013

2, pl. II, figs. 10–12, pl. VI, figs. 117–119.

Lineus psittacinus: Bürger (1895), p. 620; Gibson (1995), pp. 342, 401.

Diagnosis. A *Notospermus* with white rings either restricted to anterior body or distributed down to posterior body; green cephalic band either deeply or shallowly bisected by forward indentation of white cephalic band; posterior end of body pointed, intact specimen with caudal cirrus. Partial 513-bp 16S sequences amplified using the primer pair

5'-CGCCTGTTTATCAAAAACAT-3' (16sar-L; Palumbi et al., 1991) and 5'-CCGGTCTGAACTCAGATCACGT-3' (16sbr-H; Palumbi et al., 1991), when aligned along with the ones from other congeners, possess insertions at positions 44 and 45, represented by T and C, respectively; deletion at position 237; A at positions 249 and 251; C at positions 321 and 358; and A at position 457, where A stands for adenine, C for cytosine, and T for thymine.

Material examined. NMA 4721, remaining tissue of

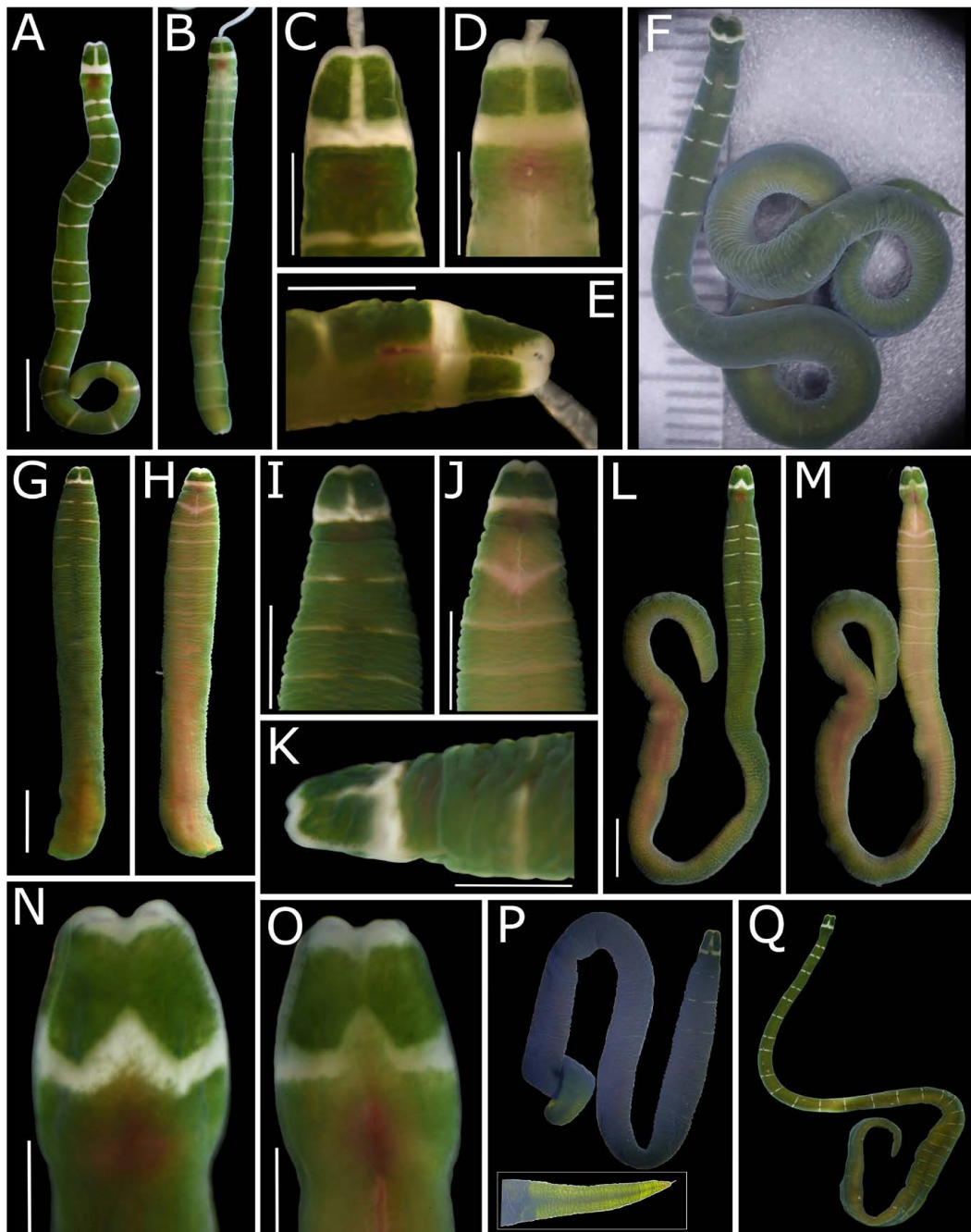


Fig. 1. *Notospermus psittacinus* (Bürger, 1890) comb. nov. collected in Mindanao, Philippines (A–Q). NMA 4721, entire specimen (posterior end cut) in dorsal (A) and ventral (B) views, magnification of the head in dorsal (C), ventral (D), and lateral (E) views; NMA 4722, whole specimen in dorsal (F) view; NMA 4723, entire specimen (posterior end cut) in dorsal (G), and ventral (H) views, magnification of the head in dorsal (I), ventral (J), and lateral (K) views; NMA 4727, entire specimen in dorsal (L) and ventral (M) views; magnification of the head in dorsal (N) and ventral (O) views; NMA 4725, entire specimen in dorsal view (inset its posterior end showing a caudal cirrus) (P); photographed specimen in dorsal view (Q) obtained from Brgy. Pagahan, Initao, Misamis Oriental. Scale bars: 5 mm (A, G, L), 3 mm (I, J), 1 mm (C, D, E, N, O).

entire specimen after DNA extraction and preserved in 99% ethanol, Brgy. Poblacion (8.4258°N, 124.2865°E), Naawan, Misamis Oriental, on 8 March 2024, by Jamael Abato; NMA 4722, Brgy. Poblacion (8.6219°N, 126.0966°E), Lianga, Surigao del Sur, on 21 November 2023, by April Joy Jovita and Ahmed Menioria; NMA 4723, Brgy. Vinapor (9.0916°N, 125.2158°E), Carmen, Agusan del Norte, on 14 March 2024, by Jamael Abato and Nick Burias; NMA 4724, Brgy.

Poblacion (8.4258°N, 124.2865°E), Naawan, Misamis Oriental, on 12 February 2024, by Caryl Kaye Awa and Alexc Mae Niez; NMA 4725, collection site and collectors same as NMA 4724, on 4 March 2024; NMA 4726 and 4727, Brgy. Poblacion (8.6219°N, 126.0966°E), Lianga, Surigao del Sur, on 17 March 2024, by Jamael Abato, Laurence Calagui, and Shirlamaine Irina Calagui. Additional specimens: one specimen, photographed only, Brgy. Poblacion

(8.4266°N, 124.2860°E), Naawan, Misamis Oriental, on 4 March 2024, by Caryl Kaye Awa and Allexc Mae Niez; two specimens, photographed only, Brgy. Pagahan (8.4665°N, 124.2874°E; 8.4663°N, 124.2876°E), Initao, Misamis Oriental, on 27 April 2024, by Caryl Kaye Awa and Allexc Mae Niez. All specimens were collected under intertidal rocks or in rock crevices/holes, submerged in water.

Description. Body greenish in color, dorsally darker (Fig. 1A, F, G, L, P, Q), ventrally lighter, pale green to light brownish green in color (Fig. 1B, H, M), 22–230 mm in length ($n = 7$, some specimens lost their posterior end), 1.3–4 mm in width ($n = 7$), with white rings encircling the head and body, 5–37 in number ($n = 7$) (Fig. 1A, B, F, G, H, L, M, P, Q); first ring in cephalic region widest, completely encircling head, situated at antero-posteriorly middle portion of cephalic slits (Fig. 1C, D, E, I, J, K, N, O); succeeding body rings equally thinner, regularly distributed throughout body (Fig. 1A, B, Q) or limited only to anterior body (Fig. 1F, G, H, L, M, P); dorsally, rings not completely continuous (Fig. 1A, F, G, L, P, Q). Head demarcated from body; anterior edges colorless, rectangular at dorsal view; dorsally, green area anterior to first white ring nearly completely separated into two halves by white, mid-longitudinal band extending anteriorly from first white ring reaching almost to anterior edge of

green area (Fig. 1C, P), first white ring sometimes anteriorly extending shortly to the green area of the head (Fig. 1F, G, Q), anteriorly twisted shortly resembling an inverted “V” (Fig. 1L, N) and divided into halves by a greenish forward indentation of the green band behind it (Fig. 1P); ventrally, green area rectangular, with anterior edge not reaching proboscis pore, making ventral tip of head colorless, completely divided/bisected at mid by less-defined colorless longitudi-

Table 2. Uncorrected genetic distance (%) between specimens of *Notospermus psittacinus* (Bürger, 1890) comb. nov. based on partial sequences of cytochrome c oxidase subunit I gene (615 bp).

<i>Notospermus psittacinus</i> comb. nov.	1	2	3	4	5	6
1. NMA 4721						
2. NMA 4722	1.63					
3. NMA 4723	0	1.63				
4. NMA 4724	1.46	0.81	1.46			
5. NMA 4725	1.63	1.63	1.63	2.12		
6. NMA 4726	1.79	1.14	1.79	0.32	2.12	
7. NMA 4727	1.79	1.14	1.79	0.32	2.12	0

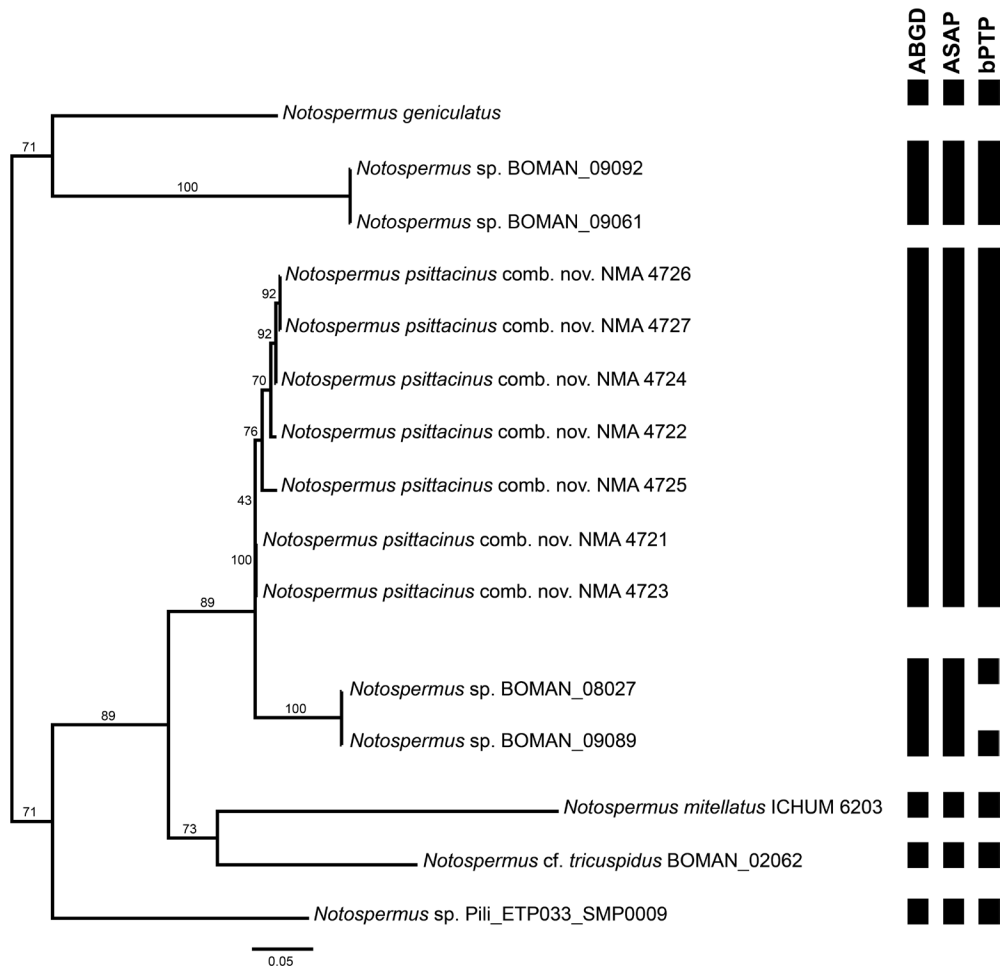


Fig. 2. Unrooted maximum likelihood tree based on the COI sequences (615 bp) of *Notospermus psittacinus* (Bürger, 1890) comb. nov. Number on each branch represents IQ-TREE ultrafast bootstrap value. Vertical bars indicate species delimitation in ABGD, ASAP, and bPTP.

nal band extending forward from first white ring (Fig. 1D), ventral colorless longitudinal band extending from first white ring sometimes absent (Fig. 1J), or twisted forward as

Table 3. Uncorrected genetic distance (%) between *Notospermus annulatus* (Grube, 1840) and *Notospermus psittacinus* (Bürger, 1890) comb. nov. based on partial sequences of 16S ribosomal RNA gene (519 bp).

<i>Notospermus</i> species	1	2	3	4	5	6
1. <i>N. annulatus</i>						
2. <i>N. psittacinus</i> comb. nov. NMA 4721	11.59					
3. <i>N. psittacinus</i> comb. nov. NMA 4723	11.39	0.59				
4. <i>N. psittacinus</i> comb. nov. NMA 4724	11.98	1.38	1.18			
5. <i>N. psittacinus</i> comb. nov. NMA 4725	11.98	1.57	1.38	0.59		
6. <i>N. psittacinus</i> comb. nov. NMA 4726	12.18	1.57	1.38	0.20	0.79	
7. <i>N. psittacinus</i> comb. nov. NMA 4727	12.18	1.57	1.38	0.20	0.79	0

inverted “V” with interior region speckled with light greenish pigments (Fig. 1O); brain noticeable as red mass posterior to first white ring, situated in front of anterior edge of mouth in ventral view (Fig. 1C, D). Ocelli black, numerous, distributed on dorso-lateral edges of head, visible in dorsal and lateral views from first white ring towards colorless tip of head (Fig. 1A, E), 12–30 ($n = 5$) in number on each side of the head.

Distribution. *Notospermus psittacinus* comb. nov. is known from its type locality, Ambon, Indonesia (Banda Sea) (Bürger, 1890), and in Upolu Island, Western Samoa (South Pacific Ocean) (Bürger, 1890). A new distribution record in the southern Philippine Sea in Mindanao is reported: Brgy. Poblacion, Naawan, Misamis Oriental; Brgy. Pagahan, Initao, Misamis Oriental; Brgy. Vinapor, Carmen, Agusan del Norte; and Brgy. Poblacion, Lianga, Surigao del Sur (present study).

Molecular analyses. From the 10 specimens of *Notospermus psittacinus* comb. nov., partial COI sequences were successfully generated from seven individuals. Uncorrected COI genetic p -distances among these specimens are 0%–2.12%, indicating conspecificity (Table 2). ABGD, ASAP, and bPTP treated all specimens as a single putative species (Fig. 2).

Partial 16S sequences were obtained from six specimens, with genetic p -distances ranging from 0% to 1.57%

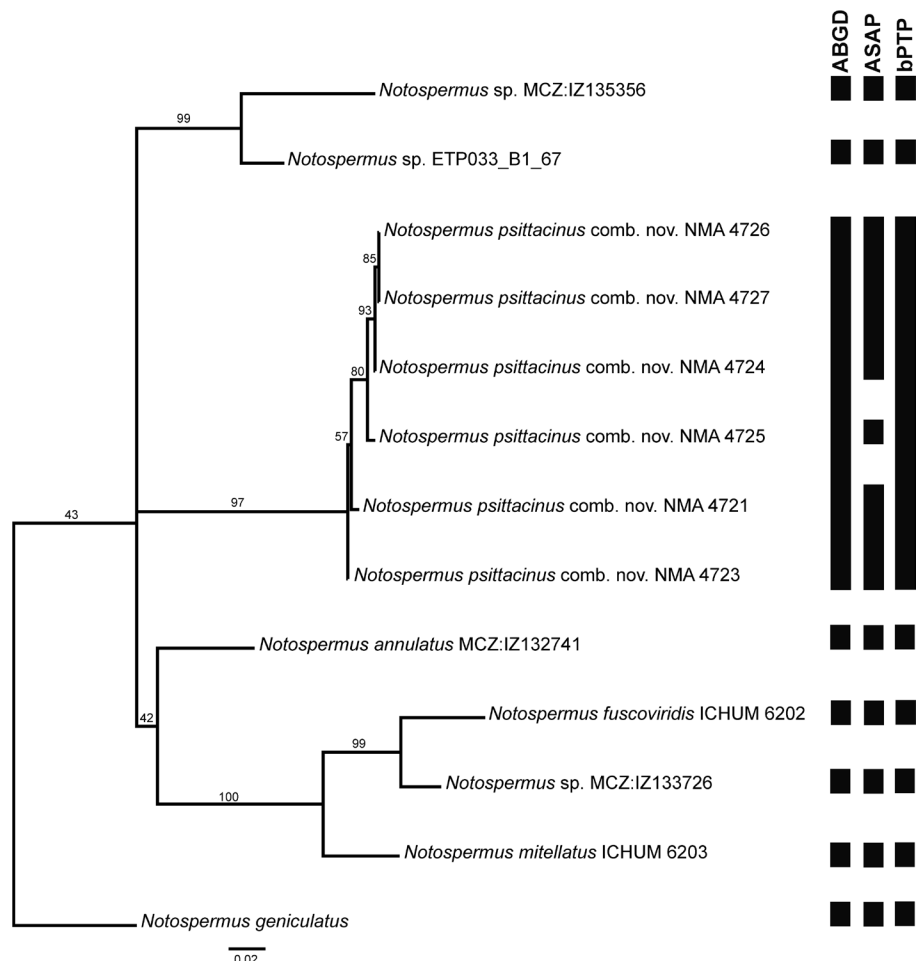


Fig. 3. Unrooted maximum likelihood tree based on the 16S sequences (519 bp) of *Notospermus psittacinus* (Bürger, 1890) comb. nov. Number on each branch represents IQ-TREE ultrafast bootstrap value. Vertical bars indicate species delimitation in ABGD, ASAP, and bPTP.

(Table 3). ABGD and bPTP treated all six specimens as a single species, while ASAP partitioned the six sequences into three hypothetical species: species 1 (NMA 4726, NMA 4727, and NMA 4724), species 2 (NMA 4725), and species 3 (NMA 4721 and NMA 4723). However, no distinguishing characteristics were identified among species groupings, except that species 2 (NMA 4725) exhibits a white cephalic band that is dorsally divided into halves by the anterior extension of the adjacent green pigmentation (Fig. 1P). Comparisons with the morphologically similar but geographically isolated *N. annulatus* revealed substantial divergence, with 16S *p*-distances of 11.39%–12.18%. All species delimitation methods based on 16S consistently separated *N. psittacinus* comb. nov. from *N. annulatus* (Fig. 3).

Due to the absence of COI data for *N. annulatus*,

COI-based comparison between the two morphologically similar species was not possible. Given its higher mutation rate compared to 16S, COI is expected to yield greater genetic distances in future comparisons. For the H3 gene, genetic *p*-distances are 5.79% between *N. annulatus* and *N. psittacinus* comb. nov., and 0% among the seven sequenced specimens of the latter species.

A phylogenetic analysis based on multiple gene markers across *Notospermus* species indicates that *N. psittacinus* comb. nov. forms a sister taxon relationship with *Notospermus* sp. BOMAN_08027/09089 with 0.99 BI posterior probability, 86% UFBoot, and 55% BS (Fig. 4). *Notospermus annulatus* falls outside this clade, highlighting its distinction from *N. psittacinus*, although its precise phylogenetic position within the genus *Notospermus* remains unresolved. The results of

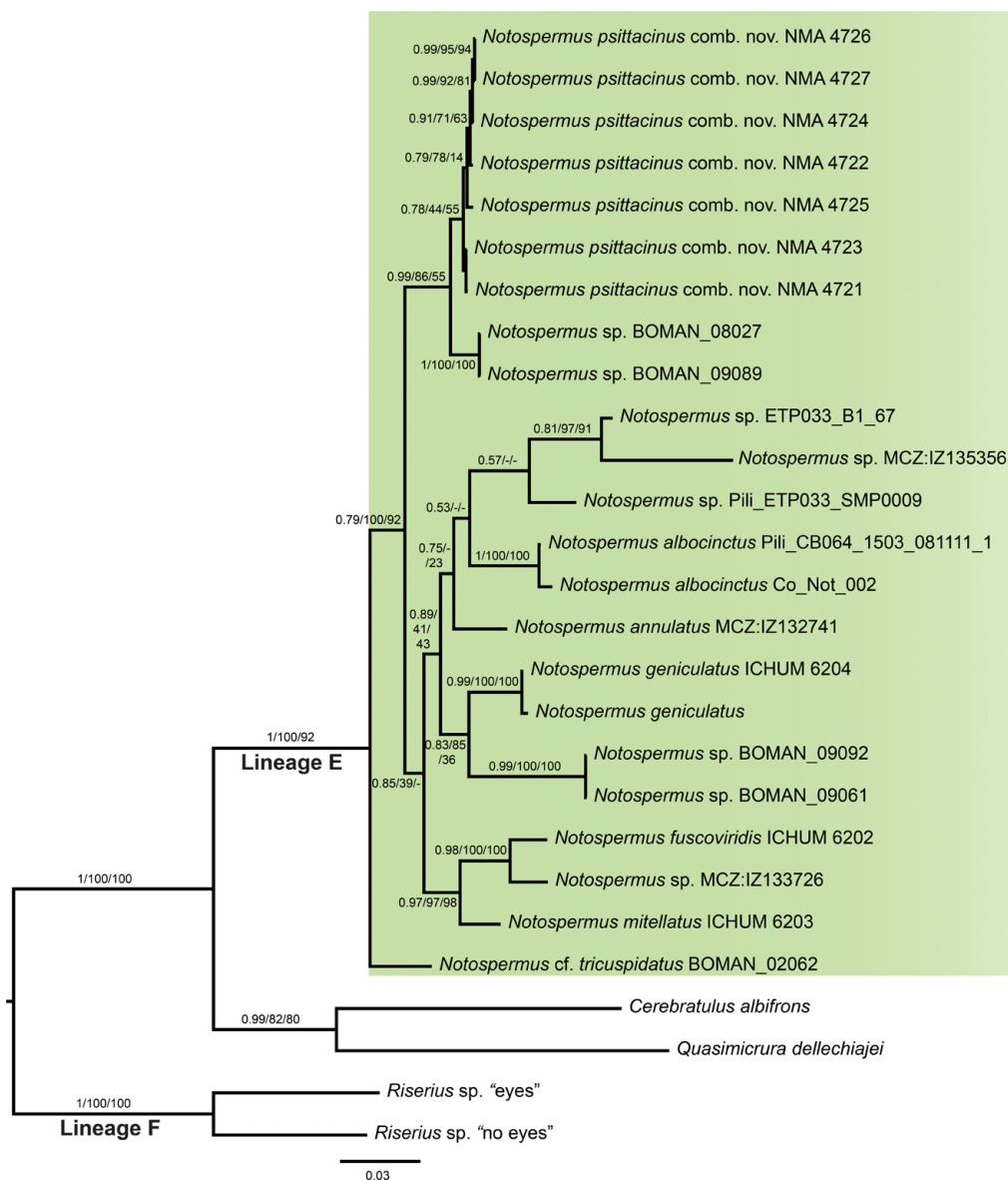


Fig. 4. Rooted maximum likelihood phylogenetic tree reconstructed in IQ-TREE showing the position of *Notospermus psittacinus* (Bürger, 1890) comb. nov. based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers on each branch represent branch support—Bayesian posterior probability (PP)/IQ-TREE maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). *Riserius* spp. serve as the outgroup in the analysis.

the molecular analyses indicate that *N. annulatus* from the Mediterranean Sea is distinct from *N. psittacinus* comb. nov. from the western Pacific.

Our phylogenetic data show that the genus *Notospermus* is monophyletic (1.00 PP/100 UFBoot/92 BS), but the intra-generic relationships remained poorly resolved.

Taxonomic remarks. Kajihara et al. (2016) investigated the occurrence of green-bodied heteronemerteans in the Indo–West Pacific and listed 20 nominal species, classified into 11 morphological forms. Forms 5 and 6 comprised of greenish heteronemerteans with white rings in the body. Form 5 species *Lineus boutani* (Joubin, 1893) is morphologically distinct from the Philippine *Notospermus* by having blue stripes on the ventral surface. Species included in Form 6 were *Lineus psittacinus* (type locality: Indonesia); *N. mitellatus* (type locality: Japan); and *Micrura tridacnae* Gibson, 1981 (type locality: Australia). From these nominal species, the Philippine *Notospermus* closely matches the external features of *L. psittacinus*, except that Bürger (1890) did not provide an explicit description of the shape of the dorsal body rings. In his illustration of a fixed *L. psittacinus* (pl. I, fig. 2), the anterior body shows dorsally connected white rings. A similar appearance was observed in one of our anaesthetised specimens (Fig. 1A). However, careful examination, particularly of living individuals, revealed that these dorsal connections are not actually present. In the anaesthetised state, or when the worm is constricted, the dorsal rings may appear connected by thin lines bridging the opposite pairs of the dorsal rings. This feature is evident in a smaller specimen, whereas larger specimens clearly display unconnected dorsal rings. Because of the observed morphological similarities between our specimens and *L. psittacinus*, as well as the geographical proximity of their collection sites (approximately 1300 km between Ambon, Indonesia and Mindanao, Philippines), we identify our specimens as the former and transfer the species to the genus *Notospermus* based on morphological and phylogenetic data, applying the name *Notospermus psittacinus* comb. nov.

Notospermus psittacinus comb. nov.-like heteronemerteans were also recorded in New Zealand (iNaturalist, 2025a, b). This may indicate that the species is distributed throughout the Indo–West Pacific region, including Indonesia, the Philippines, New Zealand, and Western Samoa. Our conservative identification of the present Philippine material relied primarily on its similarity in body coloration to the original description. To further evaluate the conspecificity among *N. psittacinus* comb. nov. from Indonesia, the Philippines, and New Zealand, collection of fresh specimens of *N. psittacinus* comb. nov. from these localities and generation of partial sequences of its COI and 16S gene are necessary. This will confirm their taxonomic identities in the future.

Notospermus psittacinus comb. nov. closely resembles the greenish *N. annulatus* (Fig. 5) in overall appearance, particularly in the shape of the thick white cephalic band and the pattern of the white body rings. However, the two look-alike species are geographically isolated and phylogenetically distinct within the genus *Notospermus*, preventing us from applying the name *N. annulatus* to the greenish Philippine *Notospermus*. The name *N. annulatus* should be applied to the greenish *Notospermus* population with dorsally non-connected white body rings in the Mediterranean Sea (Joubin, 1894; Herrera-Bachiller et al., 2015; Kahrić et al., 2022), whereas *N. psittacinus* comb. nov. should be used for the greenish heteronemertean with dorsally discontinuous white body rings occurring in Indo-West Pacific waters. DNA sequence data are necessary to accurately differentiate these two species, as well as other greenish and white-ringed heteronemerteans.

Although most described *Notospermus* (excluding the present *N. psittacinus*) can be readily identified morphologically, molecular data remain essential in the taxonomy of the genus for two reasons: *i*) a single species may exhibit considerable external pigmentation variation (e.g., *N. albobittatus* [Stimpson, 1855] in Chernyshev [2016]; *N. geniculatus* in Riser [1991]; Kajihara et al., 2022; Falconer, unpublished data; and *N. psittacinus* comb. nov.), and *ii*) cryptic species

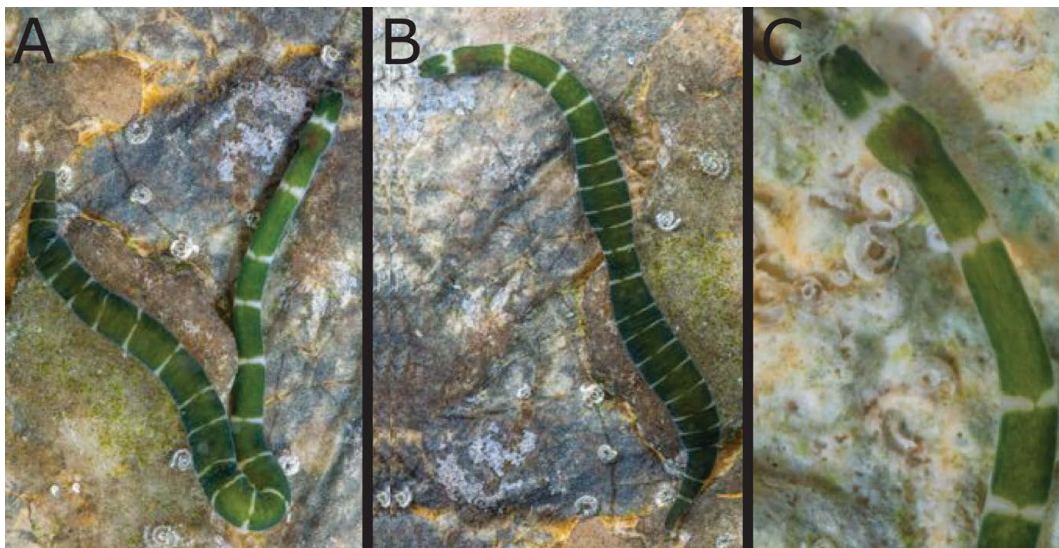


Fig. 5. *Notospermus annulatus* (Grube, 1840) (MCZ:IZ:132741) from the Mediterranean Sea (Spain). Collectors: G. Giribet and A. Herrera. Images obtained from MCZbase: The Database of the Zoological Collections, Museum of Comparative Zoology, Harvard University.

occur within the genus (e.g., *N. geniculatus* [Schwartz, pers. comm. in Kajihara et al., 2016] and *N. tricuspidatus* [Quoy and Gaimard, 1833] in Maslakova et al., 2025). Notably, geographically separated look-alike *Notospermus* have been reported, including “*N. geniculatus*” from Vietnam (Chernyshev, 2016) and *N. sp.* MoV 6812 from Australia (Falconer, unpublished data), as well as *N. tricuspidatus* from Guam and *N. cf. tricuspidatus* from Oman (Maslakova et al., 2025). COI sequence data confirmed the distinctness of the latter pair (Maslakova et al., 2025), whereas the former pair still requires molecular study to evaluate conspecificity. In this study, we showed that the true diversity of *Notospermus* can be revealed through integrative taxonomy and DNA data help to uncover cryptic diversity hidden in the genus.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

JA collected, photographed, and processed the specimens, extracted DNA, determined sequences, performed molecular analyses, wrote the first draft, and reviewed and approved the final manuscript; NAB, CKA, AJJ, and AMN collected and photographed some specimens and reviewed and approved the manuscript; NA applied for field research permit in Misamis Oriental and reviewed and approved the manuscript; AA, LC, SIC, MD, JAL, and RS applied for field research permit in Agusan del Norte and Surigao del Sur; HK improved the first draft, reviewed, and approved the final draft of the manuscript, and provided resources for the molecular analyses.

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