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*Gymnodoris nigricolor* Baba, 1960  
(Mollusca, Nudibranchia, Polyceridae)  
is a species complex

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# ***Gymnodoris nigricolor* Baba, 1960 (Mollusca, Nudibranchia, Polyceridae) is a species complex**

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## **ABSTRACT**

Molecular and morphological data on the ecologically unique dorid nudibranch *Gymnodoris nigricolor* Baba, 1960, reveals it is a species complex comprised of at least four different species. Two specimens collected in New Caledonia match the original description of *G. nigricolor* from the main islands of Japan and are assigned to this species. Additional specimens from New Caledonia are morphologically and genetically distinct and are described herein as the new species *G. boussionae* n. sp. Moreover, three specimens collected in Okinawa, Japan, are superficially similar to the New Caledonia specimens assigned to *G. nigricolor* but are morphologically and genetically different, and therefore they are assigned to the new species *G. nagoensis* n. sp. described herein. Finally, a specimen from the Marshall Islands with sequences deposited in GenBank is also distinct and constitutes a fourth species in this complex.

## **RÉSUMÉ**

*Gymnodoris nigricolor* Baba, 1960 (Mollusca, Nudibranchia, Polyceridae) est un complexe d'espèces. Les données moléculaires et morphologiques sur le nudibranche doridien *Gymnodoris nigricolor* Baba, 1960, écologiquement unique, révèlent qu'il s'agit d'un complexe composé d'au moins quatre espèces différentes. Deux spécimens collectés en Nouvelle-Calédonie correspondent à la description originale de *G. nigricolor* du Japon et sont attribués à cette espèce. D'autres spécimens de Nouvelle-Calédonie sont morphologiquement et génétiquement distincts et sont décrits ici comme la nouvelle espèce *G. boussionae* n. sp. De plus, trois spécimens collectés à Okinawa, Japon, sont superficiellement similaires aux spécimens de Nouvelle-Calédonie attribués à *G. nigricolor*, mais sont morphologiquement et génétiquement différents, et sont donc attribués à la nouvelle espèce *G. nagoensis* n. sp. Enfin, un spécimen des îles Marshall dont les séquences sont déposées dans GenBank est également distinct et constitue une quatrième espèce de ce complexe.

**KEY WORDS**  
Phylogenetics,  
biodiversity,  
New Caledonia,  
Japan,  
new species.

**MOTS CLÉS**  
Phylogénétique,  
biodiversité,  
Nouvelle-Calédonie,  
Japon,  
espèces nouvelles.

## INTRODUCTION

The genus *Gymnodoris* Stimpson, 1855 is comprised of predatory dorid nudibranchs known exclusively from the Indo-Pacific tropics (Gosliner *et al.* 2018; Knutson & Gosliner 2022). Recent studies have dramatically increased the known biodiversity of this genus, from a handful of species to 60–70 candidate species, most of which remain undescribed (Knutson & Gosliner 2022).

*Gymnodoris nigricolor* Baba, 1960 is a highly unusual species of *Gymnodoris* because of its external appearance and dietary preferences. It was originally described from Misaki, Sagami Bay, Japan (Baba 1960) and subsequently reported from several localities in the Western Pacific Ocean (e.g. Coleman 2008; Hervé 2010; Nakano 2018; Gosliner *et al.* 2018; Mehrotra *et al.* 2021) and the Indian Ocean and Red Sea (Coleman 2008; Bidgrain 2014). Morphologically, *G. nigricolor* is characterized by having a uniform bluish black color, lacking any distinctive markings or spots (Baba 1960; Nakano 2018). No other known species of *Gymnodoris* possesses a uniform black color pattern (Gosliner *et al.* 2018). Behaviorally, *G. nigricolor* is remarkable for being the only species of nudibranch found in close association with several species of gobiid fish, which in turn have a symbiotic relationship with snapping shrimp (Mulliner 1991; Williams & Williams 1986). Individuals of *G. nigricolor* apparently feed on the soft tissue of the fish fins (Osumi & Yamasu 1994, 2000), but because they do not consume the fish completely, they are considered parasitic (Osumi & Yamasu 1994; Knutson & Gosliner 2022). This is very different from other species of *Gymnodoris*, which have relatively specialized diets on various groups of heterobranch sea slugs or their egg masses (Nakano 2018; Gosliner *et al.* 2018). Due to these unique traits and apparent lack of obvious morphological variation between individuals of *G. nigricolor* from distant localities (Bidgrain 2014; Mehrotra *et al.* 2021), *G. nigricolor* is considered to be a well characterized, widespread species (Coleman 2008; Gosliner *et al.* 2018). Recently, Knutson & Gosliner (2022) sequenced a specimen identified provisionally as *G. nigricolor* from the Marshall Islands, which belongs in a clade containing several potentially undescribed species as well as *Gymnodoris impudica* (Rüppell & Leuckart, 1830), *Gymnodoris inornata* Bergh, 1880, and *Gymnodoris tuberculosa* Knutson & Gosliner, 2014. No other attempts have been made to study the systematics of this species.

In this study, we report on several specimens identified as *G. nigricolor* collected from the Koumac area of New Caledonia as part of a larger project to document the sea slug diversity of this region. We also provide new anatomical and molecular data for this species and document the existence of previously unknown diversity in this group by comparing the New Caledonia specimens with material from near the type locality in Japan.

## MATERIAL AND METHODS

### SOURCE OF SPECIMENS

For this study, 14 specimens identified as *Gymnodoris nigricolor* were collected by scuba diving or snorkeling from Koumac and Poum, New Caledonia, and the Ryūkyū Islands, Japan (see Table 1 for collection dates and voucher information). The specimens were studied using an integrative approach including both morphological and molecular methods. Sequences of representatives of all other species of *Gymnodoris* for which sequence data are available were obtained from GenBank and included in the phylogenetic analyses (Table 1).

### MORPHOLOGICAL DESCRIPTIONS

Live animals were photographed in the field individually to document their external characteristics. Two or three (if available) specimens per species were dissected under a Nikon SMZ-1000. The reproductive system was removed from the specimens to draw the various organs in detail using a camera lucida. The penises were observed under an Olympus CX-31 compound microscope and the shape of the spines was outlined with a camera lucida. The drawings were digitalized and labeled with Adobe Photoshop v24.5. The buccal mass was also dissected and soaked in a NaOH 10% solution to dissolve the surrounding tissue. Once the tissue was removed, the radula was rinsed in distilled water and mounted on a stub, flattened, and dried. The samples were coated with gold for observation under a Jeol JSM-601 scanning electron microscope (SEM).

### MOLECULAR ANALYSES

DNA extraction was conducted using a hot Chelex® (Bio-Rad, Hercules, CA) methodology. A small piece (approximately 3 mm) of tissue was dissected from the foot of each specimen and transferred to a 1.75 mL tube with 1.0 mL of Tris-EDTA (TE) buffer (10 mM Tris, 1.0 mM ethylenediaminetetraacetic acid, pH 8.0). The mix was centrifuged for 3 min and a solution with 175.0 µL of a 10% (w/v) Chelex® 100 (US Standard 100-200 mesh, sodium form) was added in the tube. The samples were heated in a water bath (56°C for 20 minutes) and a heating block (100°C for 8 minutes) and centrifuged for 3 min. The supernatant was used for PCR. DNA amplification was conducted for two mitochondrial (cytochrome oxidase subunit 1 COI and 16S rRNA) and one nuclear (Histone H3) gene fragments using PCR. The PCR master mix was composed of 2 µL of extracted DNA (supernatant), 37.25 µL of deionized water, 2.5 µL of 10 mg mL<sup>-1</sup> bovine serum albumin (BSA), 1.00 µL of 40 mM deoxynucleotide triphosphates, 1.00 µL of 10 µM primer 1, 1.00 µL of 10 µM primer 2, 0.25 µL of 5 mg mL<sup>-1</sup> of Dream Taq (Fischer Scientific, Hampton, NH), and 5.00 µL of Dream Taq PCR buffer (Fischer Scientific, Hampton, NH). Universal Histone H3 (Colgan *et al.* 1998), COI (Folmer *et al.* 1994), and 16S rRNA (Palumbi 1996) primers were used to amplify these gene fragments for each specimen. DNA amplification was confirmed by

TABLE 1.— List of specimens included in the phylogenetic and species delimitation analyses, including species name, locality, collection date, voucher number, field number, and GenBank accession numbers.

| Species                  | Locality                        | Date         | Voucher                              | Isolate      | Field #           | GenBank accession numbers |          |          |
|--------------------------|---------------------------------|--------------|--------------------------------------|--------------|-------------------|---------------------------|----------|----------|
|                          |                                 |              |                                      |              |                   | COI                       | 16S      | H3       |
| <i>G. impudica</i>       | Santo Is., Vanuatu              | 16.IX.2006   | CASIZ 179109                         | —            | FR15-OT290        | MZ382641                  | MZ409283 | MZ399423 |
| <i>G. aurita</i>         | Maricaban Is., Philippines      | 18.IV.2008   | CASIZ 177662                         | —            | —                 | MZ382621                  | MZ409256 | MZ399398 |
| <i>G. ceylonica</i>      | Dolphen Lagoon,<br>Saudi Arabia | 4.III.2013   | CASIZ 192271                         | —            | —                 | MZ382627                  | MZ409269 | MZ399409 |
| <i>G. citrina</i>        | Maui, Hawaiian Is.              | 9.X.2008     | CASIZ 180333                         | —            | CPT-08-037        | MZ382747                  | MZ409306 | MZ399537 |
| <i>G. alba</i>           | Nelson Bay, Australia           | —            | MNCN 15.05/55472                     | —            | —                 | JX274101                  | JX274063 | —        |
| <i>G. inornata</i>       | Viti Levu Is., Fiji             | 6.X.2001     | UFIZ 425814                          | —            | —                 | —                         | MZ409292 | MZ399432 |
| <i>G. striata</i>        | Malo Is., Vanuatu               | 2.X.2006     | CASIZ 178313                         | —            | VM45-OT655        | MZ382769                  | MZ409417 | MZ399559 |
| <i>G. okinawae</i>       | Kwajalein Atoll, Marshall Is.   | 10.VI.2012   | CASIZ 193489                         | —            | —                 | MZ382730                  | MZ409379 | MZ399520 |
| <i>G. nigricolor</i>     | Koumac, New Caledonia           | 27.IX.2018   | MNHN-IM-2019-26208 JD67              | KR636-AV0615 | PQ163184          | PQ158497                  | PQ136592 |          |
| <i>G. nigricolor</i>     | Koumac, New Caledonia           | 20.IX.2018   | MNHN-IM-2019-26209 JD27              | KR224-MP0205 | PQ163185          | PQ158498                  | PQ136593 |          |
| <i>G. subornata</i>      | Kwajalein Atoll, Marshall Is.   | 1.VIII.2011  | CASIZ 187858                         | —            | —                 | MZ382776                  | MZ409424 | MZ399562 |
| <i>G. pattani</i>        | Songkla Lake, Thailand          | 5-6.X.2012   | CASIZ 190606                         | —            | —                 | MZ382661                  | MZ409302 | MZ399443 |
| <i>G. brunnea</i>        | Mabini, Philippines             | 4.V.2011     | CASIZ 185966                         | —            | —                 | KJ396779                  | MZ409258 | MZ399400 |
| <i>G. pseudobrunnea</i>  | Mabini, Philippines             | 4.V.2011     | CASIZ 185948                         | —            | —                 | MZ382662                  | MZ409303 | MZ399444 |
| <i>G. tuberculosa</i>    | Madang,<br>Papua New Guinea     | 28.XI.2012   | CASIZ 191461                         | —            | PR121-<br>PCA0482 | MZ382780                  | MZ409429 | MZ399570 |
| <i>G. nagoensis</i>      | Okinawa, Japan                  | 31.V.2012    | NSMT Mo79540                         | JP1          | —                 | PQ163187                  | —        | —        |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 4.XI.2019    | MNHN-IM-2019-26201 JD10              | KR906-AV0728 | PQ163188          | PQ158500                  | PQ136595 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 27.IX.2018   | MNHN-IM-2019-26204 JD23              | KR636-AV0564 | PQ163189          | PQ158501                  | PQ136596 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 6.IX.2018    | MNHN-IM-2019-26203 JD35              | KR301-AV0094 | PQ163190          | PQ158504                  | PQ136597 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 26.IX.2018   | MNHN-IM-2019-26205 JD39              | KR636-AV0582 | PQ163191          | PQ158502                  | PQ136598 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 26.IX.2018   | MNHN-IM-2019-26202 JD68              | KR636-LC0267 | PQ163192          | PQ158503                  | PQ136599 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 26.IX.2018   | MNHN-IM-2019-26206 JD41              | KR633-AV0523 | PQ163193          | PQ158505                  | PQ136600 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 26.IX.2018   | MNHN-IM-2019-26200 JD43              | KR636-AV0562 | PQ163194          | PQ158506                  | PQ136601 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 26.IX.2018   | MNHN-IM-2019-26207 JD44              | KR636-AV0566 | PQ163195          | —                         | PQ136602 |          |
| <i>G. boussionae</i>     | Koumac, New Caledonia           | 28.II.2019   | MNHN-IM-2019-26199 JD51              | KR252-MP0426 | PQ163196          | —                         | PQ136603 |          |
| <i>G. boussionae</i>     | Poum, New Caledonia             | 18.VI.2023   | MNHN-IM-2019-26524 JD114 ABC04-KG021 | —            | PQ163197          | —                         | —        |          |
| <i>G. cf. alba</i>       | Kekepa Is., Hawaiian Is.        | 30.V.2017    | UFIZ 508508                          | —            | —                 | MW278633                  | —        | —        |
| <i>G. cf. nigricolor</i> | Kwajalein Atoll, Marshall Is.   | 27.V.2013    | CASIZ 193492                         | —            | —                 | MZ382631                  | MZ409273 | MZ399413 |
| <i>G. sp. A</i>          | Kwajalein Atoll, Marshall Is.   | 9.III.2013   | CASIZ 193501                         | —            | —                 | MZ382719                  | MZ409368 | MZ399509 |
| <i>G. sp. AA</i>         | Mabini, Philippines             | 30.IV.2011   | CASIZ 185958                         | —            | —                 | MZ382722                  | MZ409371 | MZ399512 |
| <i>G. sp. AB</i>         | Mabini, Philippines             | 19.V.2013    | CASIZ 192457                         | —            | —                 | MZ382724                  | MZ409373 | MZ399514 |
| <i>G. sp. B</i>          | Mabini, Philippines             | 18.III.2008  | CASIZ 177405                         | —            | —                 | MZ382725                  | MZ409374 | MZ399515 |
| <i>G. sp. C</i>          | Queensland, Australia           | 31.X.2012    | CASIZ 190943                         | —            | —                 | MZ382726                  | MZ409375 | MZ399516 |
| <i>G. sp. D</i>          | Maricaban Is., Philippines      | 21.IV.2008   | CASIZ 177708                         | —            | —                 | MZ382728                  | MZ409377 | MZ399518 |
| <i>G. sp. E</i>          | Madang, Papua New<br>Guinea     | 23.XI.2012   | CASIZ 191407                         | —            | PS023-<br>PCA0428 | MZ382731                  | MZ409380 | MZ399521 |
| <i>G. sp. F</i>          | Madang, Papua New<br>Guinea     | 30.XI.2012   | CASIZ 191485                         | —            | PR141-<br>PCA0506 | MZ382733                  | MZ409382 | MZ399523 |
| <i>G. sp. G</i>          | Abulad Is., Saudi Arabia        | 10.III.2013  | CASIZ 192261                         | —            | —                 | MZ382734                  | MZ409383 | MZ399524 |
| <i>G. sp. H</i>          | Espiritu Santo Is., Vanuatu     | 25.IX.2006   | CASIZ 178774                         | —            | NR25-OT531        | MZ382735                  | MZ409384 | MZ399525 |
| <i>G. sp. I</i>          | Madang, Papua New<br>Guinea     | 13.XI.2012   | CASIZ 191191                         | —            | PR028-<br>PCA0212 | MZ382737                  | MZ409386 | MZ399527 |
| <i>G. sp. J</i>          | Pamalican Is., Philippines      | 11.VI.2004   | CASIZ 176546                         | —            | R38-OT471         | MZ382738                  | MZ409387 | MZ399528 |
| <i>G. sp. K</i>          | Madang, Papua<br>New Guinea     | 11.XI.2012   | CASIZ 191128                         | —            | PR018-<br>PCA0149 | MZ382739                  | MZ409388 | MZ399529 |
| <i>G. sp. L</i>          | Madang,<br>Papua New Guinea     | 12.XI.2012   | CASIZ 191146                         | —            | PM012-<br>PCA0167 | MZ382740                  | MZ409389 | MZ399530 |
| <i>G. sp. M</i>          | Kwajalein Atoll, Marshall Is.   | 14.VIII.2011 | CASIZ 187853A                        | —            | —                 | MZ382742                  | MZ409391 | MZ399532 |
| <i>G. sp. N</i>          | Marka Is., Saudi Arabia         | 6.III.2013   | CASIZ 192273C                        | —            | —                 | MZ382744                  | MZ409393 | MZ399534 |
| <i>G. sp. O</i>          | Kwajalein Atoll, Marshall Is.   | 30.VII.2012  | CASIZ 193502                         | —            | —                 | MZ382745                  | MZ409394 | MZ399535 |
| <i>G. sp. P</i>          | Chek Jawa, Singapore            | —            | ZRC MOL7383                          | —            | —                 | MZ382746                  | MZ409395 | MZ399536 |
| <i>G. sp. Q</i>          | Pattani, Thailand               | II.2011      | CASIZ 188013                         | —            | —                 | MZ382748                  | MZ409396 | MZ399538 |
| <i>G. sp. R</i>          | Lizard Is., Australia           | VI-VIII.2013 | CASIZ 193021                         | —            | —                 | MZ382751                  | MZ409399 | MZ399541 |
| <i>G. sp. S</i>          | Maui, Hawaiian Is.              | 15.VI.2011   | CPIC 00319                           | —            | —                 | MZ382753                  | MZ409401 | MZ399543 |
| <i>G. sp. T</i>          | Double Reef, Guam               | 16.VII.2003  | UFIZ 305252                          | —            | —                 | —                         | MZ409402 | MZ399544 |
| <i>G. sp. U</i>          | Mabini, Philippines             | 1.V.2011     | CASIZ 185961                         | —            | —                 | MZ382755                  | MZ409403 | MZ399545 |
| <i>G. sp. V</i>          | Madang, Papua New<br>Guinea     | 26.XI.2012   | CASIZ 191435                         | —            | PR109-<br>PCA0456 | MZ382758                  | MZ409406 | MZ399548 |
| <i>G. sp. W</i>          | Mabini, Philippines             | 14.V.2011    | CASIZ 185955                         | —            | —                 | MZ382762                  | MZ409410 | MZ399552 |
| <i>G. sp. X</i>          | Mabini, Philippines             | 14.V.2011    | CASIZ 176780                         | —            | —                 | MZ382764                  | MZ409412 | MZ399554 |
| <i>G. sp. Y</i>          | Echizen, Japan                  | 9.V.2009     | CASIZ 188562                         | —            | —                 | MZ382766                  | MZ409414 | MZ399556 |
| <i>G. sp. Z</i>          | Mabini, Philippines             | 24.V.2011    | CASIZ 185983A                        | —            | —                 | MZ382768                  | MZ409416 | MZ399558 |
| <i>G. sp.</i>            | Madang,<br>Papua New Guinea     | 8.XII.2012   | CASIZ 191610                         | —            | PR207-<br>PCA0631 | MZ382651                  | MZ409294 | MZ399434 |

Table 1. — Continuation.

| Species              | Locality                   | Date         | Voucher       | Isolate | Field #      | GenBank accession numbers |          |          |
|----------------------|----------------------------|--------------|---------------|---------|--------------|---------------------------|----------|----------|
|                      |                            |              |               |         |              | COI                       | 16S      | H3       |
| G. sp. 3             | Madang, Papua New Guinea   | 11.XII.2012  | CASIZ 190789  | PR229-  | PCA0690      | MZ382665                  | MZ409308 | MZ399452 |
| G. sp. 8             | Thuwal, Saudi Arabia       | 15.III.2013  | CASIZ 192267  | -       | -            | MZ382670                  | MZ409313 | MZ399456 |
| G. sp. 9             | Madang, Papua New Guinea   | 11.XII.2012  | CASIZ 190801  | PR237-  | PCA0702      | MZ382673                  | MZ409316 | MZ399459 |
| G. sp. 10            | Santo Is., Vanuatu         | 11.IX.2006   | CASIZ 178402  | -       | ED02-OT48    | MZ382677                  | MZ409321 | MZ399463 |
| G. sp. 11            | Madang, Papua New Guinea   | 12.XII.2012  | CASIZ 190811  | PR240-  | PCA0712      | MZ382680                  | MZ409324 | MZ399466 |
| G. sp. 12            | Queensland, Australia      | 4.IX.1997    | QM MO60916    | -       | -            | -                         | MZ409326 | -        |
| G. sp. 14            | Queensland, Australia      | 23.IX.2012   | CASIZ 190737  | -       | -            | MZ382682                  | MZ409327 | MZ399468 |
| G. sp. 15            | Madang, Papua New Guinea   | 13.XI.2012   | CASIZ 191187  | PR028-  | PCA0208      | MZ382684                  | MZ409329 | MZ399470 |
| G. sp. 16            | Madang, Papua New Guinea   | 12.XII.2012  | CASIZ 190838  | -       | PR245-PCA739 | MZ382687                  | MZ409332 | MZ399473 |
| G. sp. 17            | Maricaban Is., Philippines | 19.V.2011    | CASIZ 185953  | -       | -            | MZ382688                  | MZ409333 | MZ399474 |
| G. sp. 18            | Mabini, Philippines        | 4.V.2011     | CASIZ 185980  | -       | -            | MZ382689                  | MZ409334 | MZ399475 |
| G. sp. 23            | Panglao Is., Philippines   | 6.VII.2004   | CASIZ 181474  | -       | B42-OT1182   | MZ382694                  | MZ409340 | MZ399481 |
| G. sp. 24            | Madang, Papua New Guinea   | 12.XI.2012   | CASIZ 191158  | PR024-  | PCA0179      | MZ382696                  | MZ409342 | MZ399483 |
| G. sp. 31            | Al Ahlam, Saudi Arabia     | 2.III.2013   | CASIZ 192275  | -       | -            | MZ382697                  | MZ409343 | MZ399484 |
| G. sp. 33            | Queensland, Australia      | 16.XII.2012  | CASIZ 190735  | -       | -            | MZ382698                  | MZ409344 | MZ399485 |
| G. sp. 34            | Maricaban Is., Philippines | 21.IV.2008   | CASIZ 177704  | -       | -            | MZ382699                  | MZ409345 | MZ399486 |
| G. sp. 38            | Mabini, Philippines        | 7.V.2011     | CASIZ 185944  | -       | -            | MZ382702                  | MZ409348 | MZ399489 |
| G. sp. 41            | Queensland, Australia      | 4.IX.1997    | QM MO65695    | -       | -            | -                         | MZ409349 | MZ399490 |
| G. sp. 44            | Madang, Papua New Guinea   | 30.VI.2004   | CASIZ 181580  | -       | M51-OT1099   | MZ382703                  | MZ409351 | MZ399491 |
| G. sp. 47            | Mabini, Philippines        | 3.V.2011     | CASIZ 185945  | -       | -            | MZ382704                  | MZ409352 | MZ399492 |
| G. sp. 48            | Mabini, Philippines        | 20.V.2011    | CASIZ 185949  | -       | -            | MZ382705                  | MZ409353 | MZ399493 |
| G. sp. 49            | Mabini, Philippines        | 17.III.2008  | CASIZ 177347  | -       | -            | MZ382706                  | MZ409354 | MZ399494 |
| G. sp. 51            | Pulau Penang, Malaysia     | 3.X.2007     | CASIZ 176781  | -       | -            | MZ382709                  | MZ409357 | MZ399497 |
| G. sp. 53            | Mabini, Philippines        | 24.V.2011    | CASIZ 185983A | -       | -            | MZ382713                  | MZ409361 | MZ399501 |
| G. sp. 54            | Pulau Tioman, Malaysia     | 4.X.2007     | CASIZ 176782  | -       | -            | MZ382714                  | -        | MZ399502 |
| G. sp. 56            | Madang, Papua New Guinea   | 3.XII.2012   | CASIZ 191513  | PR165-  | PCA0534      | MZ382718                  | MZ409366 | MZ399507 |
| G. sp. 60            | Anilao, Philippines        | 2012         | CASIZ 192472  | -       | -            | -                         | MZ409367 | MZ399508 |
| <i>Polycera atra</i> | Santa Barbara, California  | 18.VIII.2010 | CPIC 00807    | -       | -            | -                         | KF425275 | KF425289 |

running the PCR products using gel electrophoresis with ethidium bromide and inspecting them under UV light to verify the presence of bands of appropriate size (*c.* 328 bp for Histone H3, *c.* 486 bp for 16S rRNA, and *c.* 658 bp for COI). The successful PCR products were purified using Thermo Fisher Scientific Genejet purification kits, following the manufacturer protocols, and sent to Retrogen Inc. (San Diego, CA) for Sanger sequencing.

Forward and reverse sequences were assembled, edited, aligned, and concatenated using Geneious v11.1.5 (Kearse *et al.* 2012) and the MUSCLE (Edgar 2004) plug-in in Geneious. Hypervariable regions of 16S were removed manually. Bayesian inference and maximum likelihood phylogenetic analyses were conducted on the concatenated sequences (partitioned by gene) and on each gene fragment individually using the GTR + I + G model for all gene fragments as determined with Modeltest v3.7 (Posada & Crandall 1998). Bayesian inference analyses were implemented using MrBayes v3.2.1 (Ronquist *et al.* 2012) using two runs of six chains for 10 million repetitions with a sampling interval of 1 000 repetitions and burn-in of 25% removed. Maximum likelihood analyses were conducted

in raxmlGUI v1.0 (Silvestro & Michalak 2012) using the bootstrap + consensus option and the GTR+I+G model with 10 000 bootstrap repetitions. *Polycera atra* MacFarland, 1905 was used to root the resulting trees. Nodes in the resulting phylogenetic tree with posterior probabilities (PP)  $\geq 90\%$  and bootstraps values (MLB)  $\geq 70\%$  were interpreted as supported.

Two species delimitation analyses were conducted to determine the number of species in the sample using COI sequences of 11 specimens of the *G. nigricolor* species complex: 1) the Assemble Species by Automatic Partitioning (ASAP) software (Puillandre *et al.* 2021) and 2) the Bayesian Implementation of the Poisson Tree Processes (bPTP) model (Zhang *et al.* 2013). For ASAP the FASTA COI alignment was analyzed on the web app (<https://bioinfo.mnhn.fr/abi/public/asap>) using the Simple Distance (p-distances) method with the default settings. For bPTP, a COI guide tree rooted on the outgroup taxon was generated in MrBayes as described above and analyzed on the web app (<https://species.h-its.org/ptp/>) for  $5 \times 10^5$  MCMC generations, discarding 25% as burn-in and removing the outgroup before species delimitation.

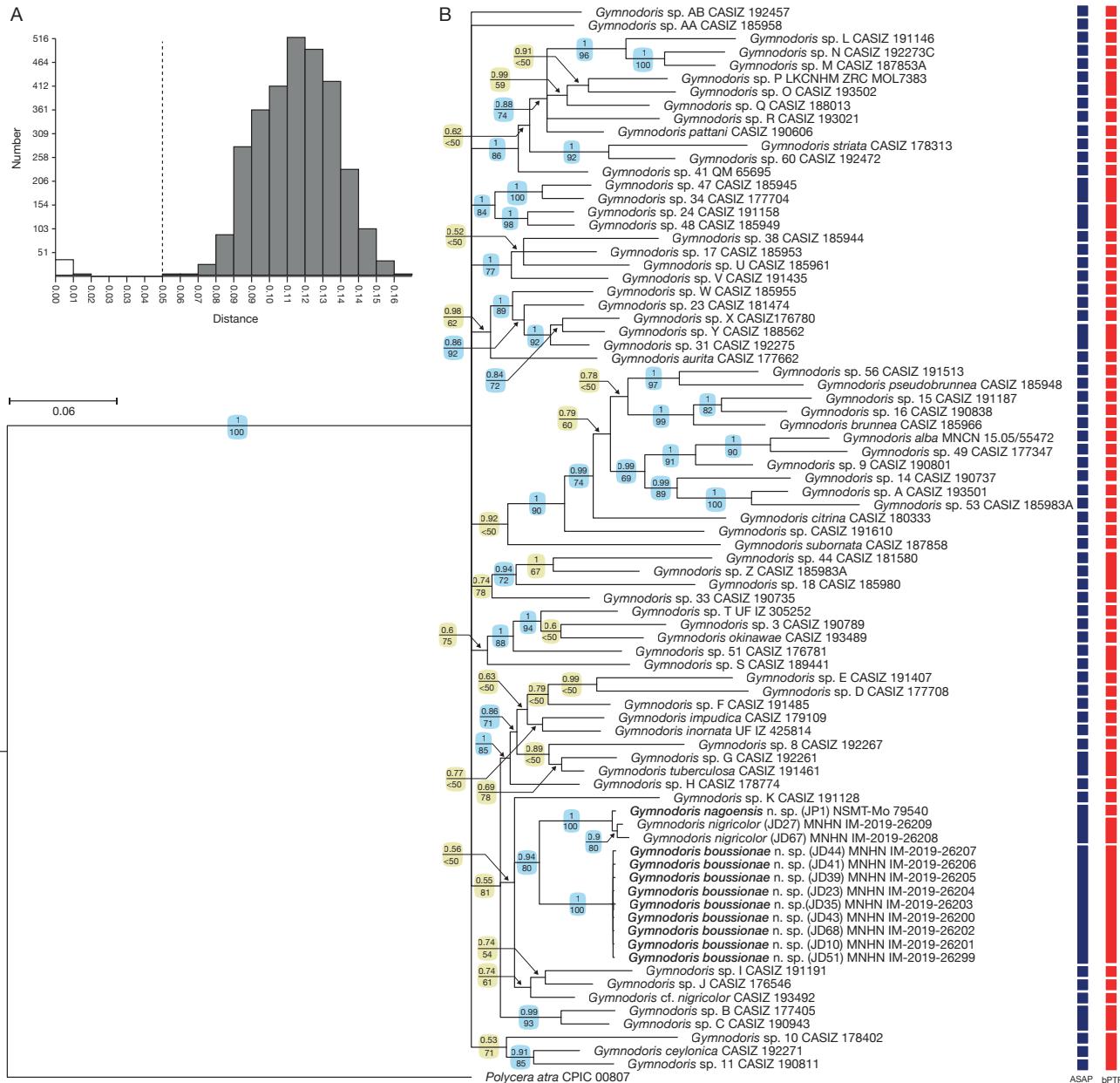


FIG. 1.— A, histogram representing the distance plot for the ASAP analysis using the COI gene showing pairwise p-distances; B, consensus phylogenetic tree of specimens sequenced based on Bayesian analysis of the three concatenated loci. Posterior probabilities from Bayesian inference (B) are given above branches and percentages corresponding to maximum likelihood bootstrap support are given below branches. Supported nodes are highlighted in blue, not supported nodes are highlighted in orange. Results of two species delimitation analyses are indicated by vertical bars.

## RESULTS

The ASAP analysis (Fig. 1A) recovered three species in the *G. nigricolor* species complex, which corresponds to the clade from New Caledonia and the clade including specimens from New Caledonia and Japan recovered in the phylogenetic analyses and the specimen identified as *G. cf. nigricolor* from the Marshall Islands (CASIZ193492). The COI genetic distances generated for the ASAP analysis are provided in Supplementary material 2. However, the bPTP recovered four species, as the specimen from Japan was recovered as a distinct species.

In the concatenated tree (Fig. 1B), the basal relationships among species of *Gymnodoris* are unresolved (polytomy), however relationships among closely related species are generally well supported. Focusing on the *G. nigricolor* species complex, the species recognized as *G. nigricolor* is monophyletic (PP: 0.9, MLB: 80) and sister to the species described herein as *G. nagoensis* n. sp. (PP: 1, MLB: 100). Members of the species recognized herein as *G. boussoniae* n. sp. also form a monophyletic group (PP: 1, MLB: 100), which is sister to the clade containing *G. nigricolor* and *G. nagoensis* n. sp. (PP: 0.94, MLB: 80). The relationships between the clade including *G. nigricolor*, *G. nagoensis* n. sp. and *G. boussoniae* n. sp. are poorly resolved.

*sionae* n. sp. and other species of *Gymnodoris* is not resolved. This includes the species identified as *G. cf. nigricolor* from the Marshall Islands (CASIZ 193492) whose relationships with other species of the *G. nigricolor* species complex are not supported.

The morphological examinations revealed consistent differences between representative specimens of three species recovered in the species delimitation analyses. In the Systematics section below these differences, including internal morphological characteristics, such as the radula and the reproductive system, and external morphological traits, are described and discussed.

#### ABBREVIATIONS

##### Institutions

|       |  |
|-------|--|
| CASIZ | California Academy of Sciences Invertebrate Zoology, San Francisco;                |
| CPIC  | California State Polytechnic University, Invertebrate Collection, Pomona;          |
| MNCN  | Museo Nacional de Ciencias Naturales, Madrid;                                      |
| MNHN  | Museum national d'Histoire naturelle, Paris;                                       |
| NSMT  | National Museum of Nature and Science, Tokyo;                                      |
| QM    | Queensland Museum Kurilpa, Brisbane;   |
| UFIZ  | University of Florida, Invertebrate Zoology, Gainesville;                          |
| ZRC   | Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore. |

##### Other abbreviations

|     |                       |
|-----|-----------------------|
| am  | ampulla;              |
| bc  | bursa copulatrix;     |
| dd  | deferent duct;        |
| fg  | female gland complex; |
| pr  | prostate;             |
| stn | station;              |
| sr  | seminal receptacle;   |
| vg  | vagina.               |

#### SYSTEMATICS

##### Class GASTROPODA Cuvier, 1797

##### Superfamily POLYCEROIDEA Alder & Hancock, 1845

##### Family POLYCERIDAE Alder & Hancock, 1845

##### Subfamily POLYCERINAE Alder & Hancock, 1845

##### Genus *Gymnodoris* Stimpson, 1855

##### *Gymnodoris nigricolor* Baba, 1960

(Figs 2A, B; 3A, B; 4A, B)

*Gymnodoris nigricolor* Baba, 1960: 72, pl. 5, figs 1A, B.

MATERIAL EXAMINED.—New Caledonia • 1 specimen 5 mm preserved length, dissected; Koumac, East of Karembe; 20°39.6'S, 164°17.5'E; 2-5 m; 27.IX.2018; Koumac 2.1 Stn KR636; isolate JD67; MNHN-IM-2019-26208 • 1 specimen 6 mm preserved length, dissected; Koumac, Channel between Tangadiou Islet and Magone; 20°33.9'S, 164°13.2'E; 7-8 m; 20.IX.2018; Koumac 2.1 Stn KR224; isolate JD27; MNHN-IM-2019-26209.

#### DESCRIPTION

Body elongate, smooth, lacking tubercles (Fig. 2A, B). Body color velvet black, with blurry neon-blue lines visible on

mantle margin under certain light conditions. Rhinophores and branchial leaves with same color as dorsum. Rhinophores short, conical, fused together at base. Rhinophoral club occupying entire visible length, with six transverse lamellae. Gill composed of 10-11 simple leaves, arranged in a circle.

Radular formula 10 × 7.1.1.0.1.1.7 in a 6 mm-long specimen (MNHN-IM-2019-26209, isolate JD27) and 9 × 6.1.1.0.1.1.6 in a 5 mm-long specimen (MNHN-IM-2019-26208, isolate JD67). Rachidian teeth absent (Fig. 3A). Innermost lateral teeth hook-shaped, with elongate cusp, 3-4 denticles (Fig. 3A). Second innermost lateral teeth broad, with wide base, two lateral flattened extensions, large, irregular, elongate central cusp (Fig. 3A). Outermost teeth similar in size, elongated, hamate with wide base, long conical cusp (Fig. 3B). Labial cuticle smooth.

Reproductive system (Fig. 4A) with pyriform ampulla connecting with both female gland complex, prostate. Prostate tubular, convoluted, with several folds, much wider than ampulla. Prostate narrowing into curved duct before expanding into elongate, convoluted deferent duct, narrower than prostate. Vagina as wide as deferent duct, mostly straight, curving before connecting directly into bursa copulatrix. Seminal receptacle small, pyriform, connecting to oval bursa copulatrix opposite to vaginal connection; uterine duct short, connected to female gland complex. Bursa copulatrix twice as large as seminal receptacle. Penis with 200-400 mm-long, elongate spines with narrow bases (Fig. 4B).

#### BIOLOGY

Original described from Japan (Baba 1960). This species was reported from New Caledonia by Hervé (2010), based on a photograph of a specimen found in Nouméa, but the identity of the specimen is unclear. The present is the first confirmed record of this species from New Caledonia where it is rare, found on sand at 7-8 m depth. The specimens were collected by direct collection while scuba diving. The feeding behavior was not observed, but specimens identified as *G. nigricolor* from Japan were reported to feed on the soft tissue of goby fish fins (Osumi & Yamasu 1994, 2000).

#### REMARKS

Baba (1960) described *G. nigricolor* based on a single specimen collected on a muddy bottom at 2 m depth in Misaki, Sagami Bay, Japan, as being “very distinct in coloring and in the shape of the radula teeth. Length 4 mm. Body smooth. Gills small, consisting of 9 plumes arranged in a semicircle open behind. Back and sole uniformly bluish black; rhinophores and gills also bluish black. Radula formula 22 × 7-8.1.0.1.7-8. First lateral broad and tricuspidate (the median cusp sharp, the lateral cusps blunt). Next laterals pyriform.”

Two specimens examined here from New Caledonia presents the same morphological features as described by Baba (1960: fig. 1A, B) for *G. nigricolor*, with a high resemblance in the radular morphology, having a truncated triangular innermost teeth with a large central conical cusp and two lateral flat cusps, and elongated hamate outermost lateral teeth. Baba's (1960) illustration of the live animal also shows the

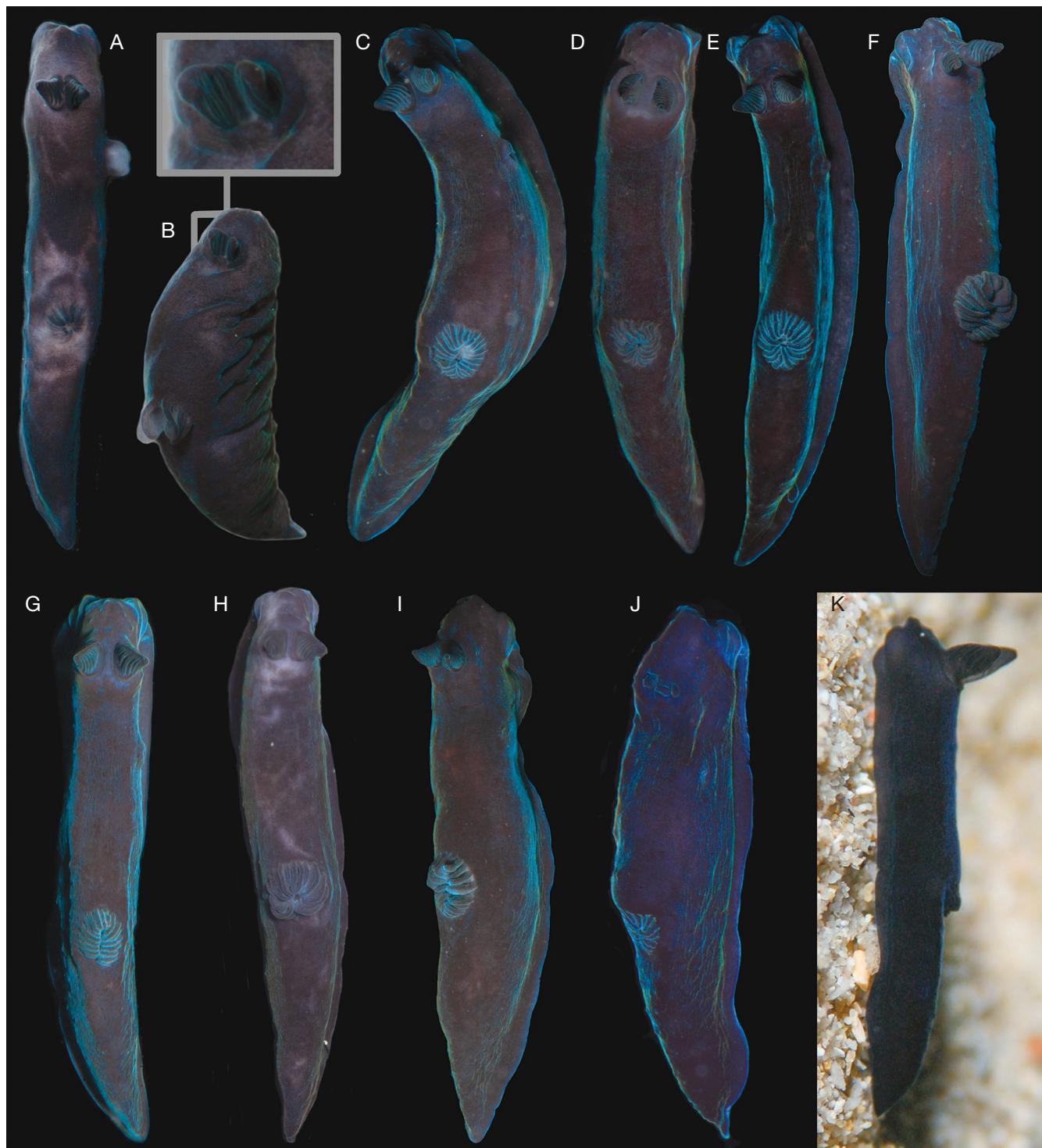


FIG. 2.—Photographs of live animals of the *Gymnodoris nigricolor* species complex: **A, B**, *Gymnodoris nigricolor* Baba, 1960, specimen MNHN-IM-2019-26208, isolate JD67 (**A**), specimen MNHN-IM-2019-26209, isolate JD27 (**B**); **C-J**, *Gymnodoris boussionae* n. sp. specimen MNHN-IM-2019-26204, isolate JD23 (**C**), specimen MNHN-IM-2019-26203, isolate JD35 (**D**), specimen MNHN-IM-2019-26206, isolate JD41 (**E**), specimen MNHN-IM-2019-26200, isolate JD43 (**F**), specimen MNHN-IM-2019-26205, isolate JD39 (**G**), specimen MNHN-IM-2019-26299, isolate JD51 (**H**), specimen MNHN-IM-2019-26207, isolate JD44 (**I**), specimen MNHN-IM-2019-26201, isolate JD10 (**J**); **K**, *Gymnodoris nagoensis* n. sp., specimen from Sunabe, Okinawa Prefecture, Japan, NSMT-Mo 79541, photo H. Tokura.

rhinophores are fused at the base. Based on these similarities we are confident the two specimens here examined belong to this species. Unfortunately, there are no other anatomical

descriptions of specimens assigned to *G. nigricolor* from other localities that could be used to compare with the material here examined.

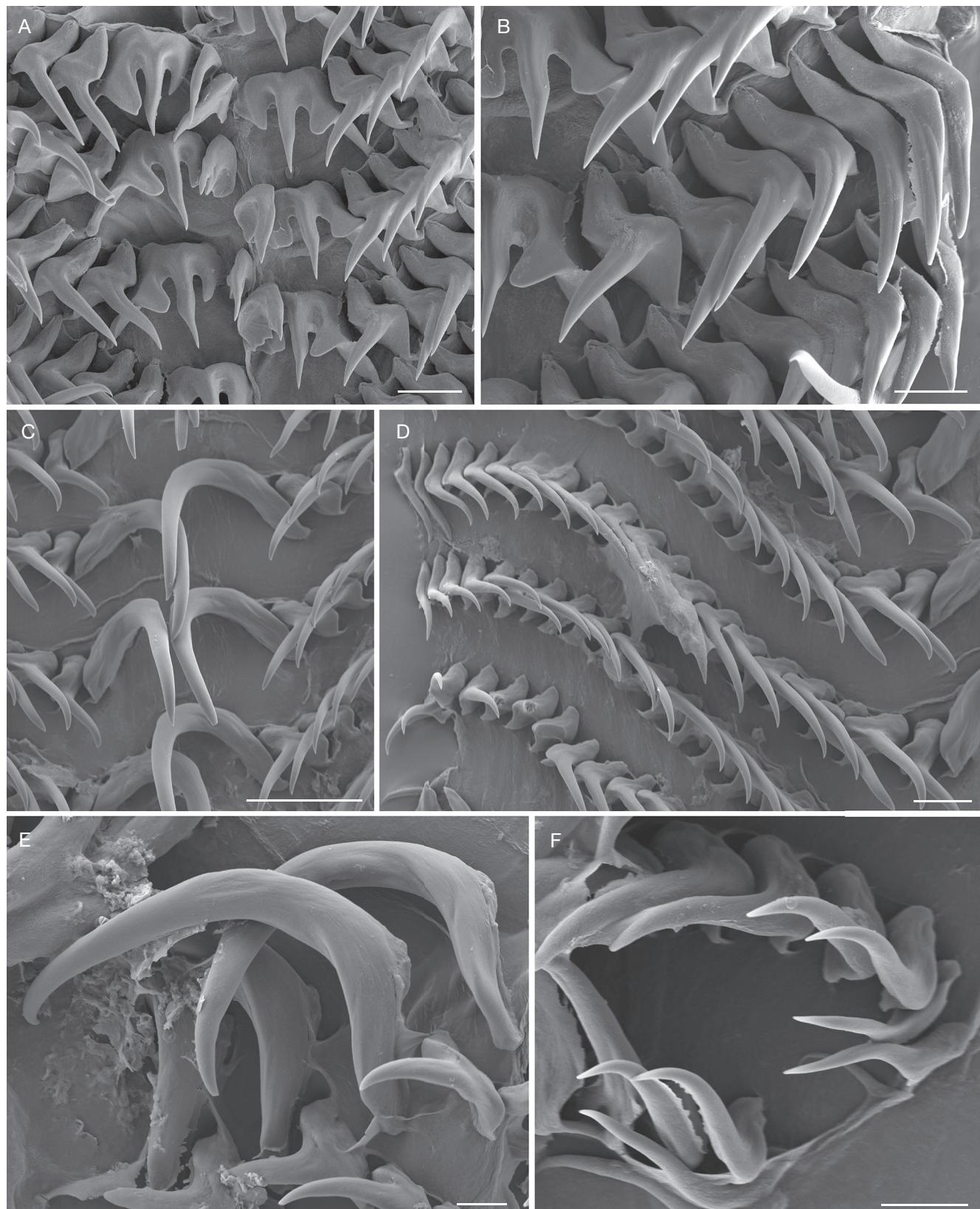


Fig. 3.— SEM of the radula of the *Gymnodoris nigricolor* species complex: **A, B**, *Gymnodoris nigricolor* Baba, 1960, specimen [MNHN-IM-2019-26209](#), isolate JD27, innermost teeth (**A**) and outer lateral teeth (**B**); **C-D**, *Gymnodoris boussoniae* n. sp., specimen [MNHN-IM-2019-26203](#), isolate JD35, innermost teeth (**C**) and outer lateral teeth (**D**); **E-F**, *Gymnodoris nagoensis* n. sp., specimen from Sunabe, Okinawa Prefecture, Japan, NSMT-Mo 79541, innermost teeth (**E**) and outer lateral teeth (**F**). Scale bars: A, 30 µm; B, D, 20 µm; C, 30 µm; E, F, 10 µm.

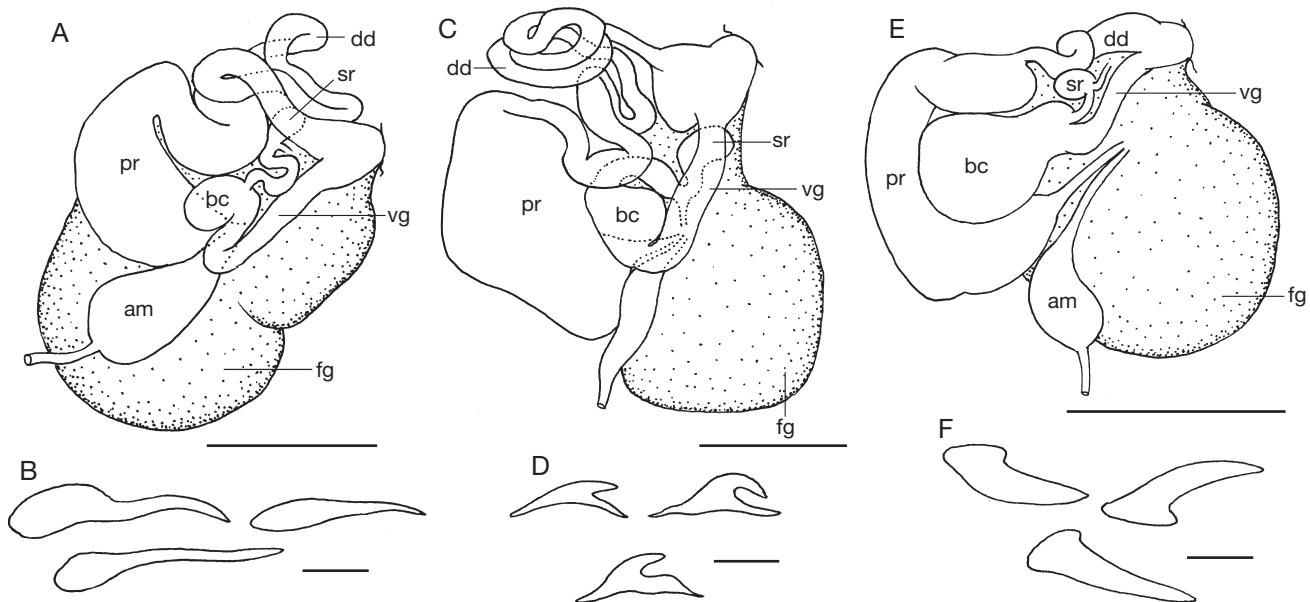


FIG. 4.— Drawings of the reproductive system of the *Gymnodoris nigricolor* species complex: **A**, **B**, *Gymnodoris nigricolor* Baba, 1960, specimen MNHN-IM-2019-26209, isolate JD27, reproductive system (**A**) and penial spines (**B**); **C**, **D**, *Gymnodoris boussionae* n. sp., specimen MNHN-IM-2019-26203, isolate JD35, reproductive system (**C**) and penial spines (**D**); **E**, **F**, *Gymnodoris nagoensis* n. sp. specimen from Sunabe, Okinawa Prefecture, Japan, NSMT-Mo 79541, reproductive system (**E**) and penial spines (**F**). Scale bars: A, C, E, 1 mm; B, D, F, 100 µm. Abbreviations: see Material and methods.

### *Gymnodoris boussionae* n. sp. (Figs 2C; 3C, D; 4C, D)

urn:lsid:zoobank.org:act:ED997B72-5312-4C15-A9B7-AC8582A9BFCF

TYPE MATERIAL. — Holotype. New Caledonia • specimen 10 mm preserved length; Koumac; East of Karembe; 20°39.1'S, 164°17.6'E; 6 m; 26.IX.2018; Koumac 2.1 Stn KR633; isolate JD41; MNHN-IM-2019-26206.

OTHER MATERIAL EXAMINED. — New Caledonia • 1 specimen 7 mm preserved length, dissected; Koumac; Reef of Infernet; 20°36.7'S, 164°14.7'E; 5 m; 6.IX.2018; Koumac 2.1 Stn KR301; isolate JD35; MNHN-IM-2019-26203 • 1 specimen 7 mm preserved length; Koumac; East of Karembe; 20°39.6'S, 164°17.5'E; 2-5 m; 27.IX.2018; Koumac 2.1 Stn KR636; isolate JD23; MNHN-IM-2019-26204 • 1 specimen 11 mm preserved length; isolate JD43; MNHN-IM-2019-26200 • 1 specimen 11 mm preserved length; isolate JD44; MNHN-IM-2019-26207 • 1 specimen 5 mm preserved length, dissected; isolate JD68; MNHN-IM-2019-26202 • 1 specimen 9 mm preserved length, dissected; isolate JD39; MNHN-IM-2019-26205 • 1 specimen 6 mm preserved length, dissected; Koumac; 20°38.4'S, 164°16.5'E; 28.II.2019; Koumac 2.2 Stn KR252; isolate JD51; MNHN-IM-2019-26199 • 1 specimen 8 mm preserved length; Koumac; 20°33.3'S, 164°12.7'E, 4 m; 4.XI.2019; Koumac 2.3 Stn KR90; isolate JD106; MNHN-IM-2019-26201 • 1 specimen 4 mm preserved length; Poum, Ouanne; 20°20.73'S, 164°1.22'E; 0 m; 11.VI.2023; ABC Poum Stn ABC04; isolate JD114; MNHN-IM-2019-26524.

ETYMOLOGY. — This species is named in honor of Magali Boussion diver and underwater videographer from New Caledonia, in appreciation for her participation in several sea slug collecting expeditions and documentation of the behavior of this species in nature.

### DESCRIPTION

Body elongate, smooth, lacking tubercles (Fig. 2D-J). Body color velvet black, with blurry neon-blue lines visible on mantle margin under certain light conditions. Rhinophores and branchial leaves with same color as dorsum. Rhinophores short, conical, not fused together at base. Rhinophoral club occupying entire visible length, with 10 transverse lamellae. Gill composed of 16-19 simple leaves, arranged in a circle.

Radular formulae 19 × 13.1.0.1.13 in a 5 mm-long specimen (MNHN-IM-2019-26202, isolate JD68), 27 × 16.1.0.1.16 in a 6 mm-long specimen (MNHN-IM-2019-26199, isolate JD51), and 29 × 18.1.0.1.18 in a 8 mm-long specimen (MNHN-IM-2019-26203, isolate JD35). Rachidian teeth absent. Innermost lateral teeth hamate, with very elongate, curved cusp, no denticles (Fig. 3C). Outer later teeth much smaller than innermost teeth, further decreasing in size gradually towards outer edge, hamate with wide base, long curved cusp (Fig. 3D). Labial cuticle smooth.

Reproductive system (Fig. 4C) with elongate, simple, narrow ampulla, connecting to both female gland, prostate. Prostate flat, almost as wide as female gland. Prostate narrowing into long, curved duct before expanding into long, convoluted deferent duct. Deferent duct narrower than ampulla at widest point. Vagina straight, wider than ampulla, curving before connecting directly into bursa copulatrix. Seminal receptacle small, elongated, connecting to oval bursa copulatrix opposite to vaginal connection; uterine duct short, connecting to female gland complex. Bursa copulatrix twice as large as seminal receptacle. Penis with 200 mm-long spines with curved cusps and long bases (Fig. 4D).

**BIOLOGY**

Possibly an endemic from New Caledonia, rare, 5 m. The specimens were collected by direct collection while scuba diving. The feeding behavior was not observed.

**REMARKS**

With the exception of *G. nigricolor* and the specimen from the Marshall Islands assigned to *G. cf. nigricolor* by Knutson & Gosliner (2022), there are no other species of *Gymnodoris* described to date with a uniformly velvet black body color (see Gosliner et al. 2018; Knutson & Gosliner 2022). Thus, *G. boussionae* n. sp. is externally clearly distinguishable from all other species of *Gymnodoris*.

There are several differences between *G. boussionae* n. sp. and *G. nigricolor* that justify the description of a new species. First, these two species are consistently recovered as distinct groups in all species delimitation analyses. Second, the radular of *G. boussionae* n. sp. is clearly different from that of *G. nigricolor*, having the innermost teeth curved, very elongate in comparison to mid-lateral teeth, whereas in *G. nigricolor* the innermost teeth are short, each tooth with a central cusp and fewer hamate outer teeth. Third, the reproductive system of *G. boussionae* n. sp. contains small penial spines (200 µm) with elongate bases and curved cusps, and a broad flattened prostate, whereas the spines of *G. nigricolor* are larger (200–400 mm) and elongate, and the prostate is tubular. Fourth, the rhinophores of *G. boussionae* n. sp. are separate at the base, but they are fused together at the base in *G. nigricolor*.

There are no morphological descriptions available of the specimen from Marshall Islands to compare the radula or reproductive system with the specimens of *G. boussionae* n. sp. here described. However, the former species is clearly distinct as it is consistently recovered in a different group in all species delimitation analyses, and it is not closely related to *G. boussionae* n. sp. in the phylogenetic analyses. Other authors are currently working on the description of the specimen from the Marshall Islands (Gosliner, pers. comm.).

*Gymnodoris nagoensis* n. sp.  
(Figs 2K; 3E, F; 4E, F)

urn:lsid:zoobank.org:act:4EAC8527-DA41-4C9A-A3DD-AB36DFDCA528

TYPE MATERIAL. — Holotype. Japan • specimen 6 mm preserved length, dissected; Okinawa Prefecture; Nago; Kouki; 26°32.8'N, 127°57.7'E; 31.V.2012; 10 m; isolate JP01; NSMT-Mo 79540.

OTHER MATERIAL EXAMINED.— Japan • 2 specimens 4 mm preserved length, dissected; Okinawa Prefecture, Ginowan, Sunabe; 26°19.4'N, 127°44.6'E; 23.II.2010; 16 m; NSMT-Mo 79541 • 1 specimen 4 mm preserved length, dissected; Kagoshima Prefecture; Amami Ōshima Island; Tebiro Beach; 28°24.1'N, 129°37.2'E; 9.III.2010; 9 m; NSMT-Mo 79542.

ETYMOLOGY. — Named after Nago, Okinawa Prefecture, Japan, the type locality of this species.

**DESCRIPTION**

Body elongate, smooth, lacking tubercles (Fig. 2K). Body color velvet black, with blurry neon-blue lines visible on mantle margin under certain light conditions. Rhinophores and branchial leaves with same color as dorsum. Rhinophores elongate, conical, not fused together at base. Rhinophoral club occupying entire visible length, with transverse five lamellae. Gill composed of 10 simple gill leaves, lacking lamellae, arranged in a circle.

Radular formulae 18 × 11.1.0.1.11 in a 4 mm-long specimen (NSMT-Mo 79541) and 18 × 12.1.0.1.12 in a 6 mm-long specimen (NSMT-Mo 79540). Rachidian teeth absent. Innermost lateral teeth hamate, with very elongate, curved cusp, no denticles (Fig. 3E). Outer later teeth much smaller than innermost teeth, further decreasing in size gradually towards outer edge, hamate with wide base, long curved cusp (Fig. 3F). Labial cuticle smooth.

Reproductive system (Fig. 4E) with simple, pyriform ampulla connecting to both female gland, prostate. Prostate tubular, curved, much narrower than female gland. Prostate narrowing into relatively convoluted duct before expanding into short deferent duct. Deferent duct narrower than ampulla at its widest point. Vagina slightly curved, as wide as deferent duct, connecting directly into bursa copulatrix. Seminal receptacle small, oval, connecting to large, nearly spherical bursa copulatrix, in expanded region near vaginal connection; uterine duct short, connecting to female gland complex. Bursa copulatrix several times as large as seminal receptacle. Penis with 200–300 mm-long, elongate spines with narrow bases, elongate cusps (Fig. 4F).

**BIOLOGY**

Found on sandy/silty bottoms at depths of 3–30 meters on Okinawa and Amami Ōshima island. When individuals of *G. nagoensis* n. sp. find a goby hole, they crawl into it. It seems that the nudibranch feeds on the fins of the goby; sometimes several individuals feed on the fins of a goby at the same time.

**REMARKS**

*Gymnodoris nagoensis* n. sp. is genetically very similar to *G. nigricolor* Baba, 1960 (Supplementary material 2; Fig. 1B) and recovered as the same species in the ASAP analysis but distinct in the bPTP analysis. Unfortunately, we were only able to amplify COI from one specimen despite multiple attempts. However, *G. nagoensis* n. sp. is anatomically different from all other species here examined. Externally, the rhinophores of *G. nagoensis* n. sp. are proportionally longer and have a reduced number of branchial leaves. The radular morphology of *G. nagoensis* n. sp. is similar to that of *G. boussionae* n. sp. but clearly distinct from that of *G. nigricolor*. The innermost teeth of *G. nagoensis* n. sp. are very elongate in comparison to mid-lateral teeth and lack denticles, whereas the innermost teeth of *G. nigricolor* are approximately the same size as the mid-lateral teeth, with truncated triangles, a large central conical cusp, and two lateral flat cusps. The teeth of *G. nagoensis* n. sp. are very similar to those of *G. boussionae* n. sp. even though

these two species are phylogenetically more distant, and their reproductive anatomy is substantially different. The reproductive anatomy of *G. nagoensis* n. sp. is unique, having large, elongate penial spines with small bases, a bursa copulatrix several times larger than the seminal receptacle and a very short deferent duct. This combination of traits is not present in *G. boussionae* n. sp. or *G. nigricolor* and clearly differentiates *G. nagoensis* n. sp. from the other two species.

## DISCUSSION

The present paper provides evidence of a cryptic sea slug species complex, in which [some] species cannot be distinguished by external characteristics. In this case, *G. boussionae* n. sp. and *G. nagoensis* n. sp. appear to be completely indistinguishable. However, *G. nigricolor* can be identified by the presence of fused rhinophores at the base and therefore could be considered pseudocryptic, which is a term used when after a molecular study is conducted, several subtle external differences among species become obvious (e.g., Krug *et al.* 2018). Most cases of cryptic speciation reported in the recent literature are represented by pseudocryptic complexes, and cases of truly cryptic speciation are more rare, and could be indicative of different evolutionary processes (ecological) resulting in cladogenesis (Churchill *et al.* 2014).

The results of the present study reveal that *Gymnodoris nigricolor* is a species complex of at least four different species. Because our sampling was limited to New Caledonia and Okinawa, and GenBank sequences from the Marshall Islands, it is possible that more biodiversity remains undiscovered. For example, Mehrotra *et al.* (2021) illustrated a specimen similar to the material of *G. nigricolor* here examined but with a white gill, which could represent yet another distinct species of the complex. The geographic range of the *G. nigricolor* species complex includes a large portions of the Indo-Pacific region from the Central Pacific Ocean (Knutson & Gosliner, 2022) to the Western Indian Ocean (Bidgrain, 2014), thus, it is likely that additional research will reveal more members of this group.

One interesting aspect of the results of this study is the fact that there is a mismatch between the radular morphology and the phylogenetic position of the studied species. Whereas *G. nigricolor* is closely related to *G. nagoensis* n. sp., to the point that the ASAP analysis cannot separate them into different species, the radular morphology of these two species is very different. This apparent convergence in radular morphology may be suggestive of dietary specialization playing an important role in the diversification of this group. With the exception of *G. nagoensis* n. sp., none of the specimens here examined have been observed feeding in the field, thus their diet and feeding behavior remains unknown. It is possible that not all members of the *G. nigricolor* species complex feed on gobby fish fins. Further research is needed to fully understand the evolution and biodiversity of this remarkable group.

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## SUPPLEMENTARY MATERIALS

**SUPPLEMENTARY MATERIAL 1.** — Consensus phylogenetic trees based on Bayesian inference and maximum likelihood analyses of the three loci separately. Posterior probabilities from Bayesian inference (Bi) and percentages corresponding to maximum likelihood bootstrap support are given. [https://doi.org/10.5852/zootaxa.2025v47a17\\_s1](https://doi.org/10.5852/zootaxa.2025v47a17_s1)

**SUPPLEMENTARY MATERIAL 2.** — COI p-distances among specimens included in the ASAP analysis. [https://doi.org/10.5852/zootaxa.2025v47a17\\_s2](https://doi.org/10.5852/zootaxa.2025v47a17_s2)