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FULL PAPER

Neotrygon yakkoei, a new bluespotted maskray (Dasyatidae) from Japan

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Abstract

Neotrygon yakkoei (Dasyatidae), a new species of bluespotted maskray from Japan, previously confused with *Neotrygon kuhlii* (Müller and Henle 1841) or *Neotrygon orientalis* Last, White and Séret 2016, is described on the basis of 35 specimens [146.4–425.2 mm disc width (DW)] from Japan. The new species is characterized by the following characteristics: greenish brown body in fresh with a single dark mask-like marking between eyes (often indistinct); fewer spots (mean 20.4 spots); small pale blue spots [its diameter 1.2–3.4% (mean 2.6%) of DW] surrounded by dark brown outer rings when fresh; and longer head length 35.9–43.4% (mean 39.1%) of DW. Molecular analysis based on 576 bp of partial COI mitochondrial gene also supports that *N. yakkoei* is a distinct species from all other congeners, except for *Neotrygon vali* Borsa 2017, which lacked genetic data. The new species is currently known only from Japan: Hokkaido (Japan Sea and Pacific sides), Wakasa Bay (Japan Sea), the Izu and Ogasawara islands, the Pacific coast of Japan from Sagami Bay to southern Kyushu, and the Osumi, Amami, Okinawa, and Yaeyama islands.

Keywords Taxonomy · *Neotrygon kuhlii* species complex · *Neotrygon orientalis* · *Neotrygon varidens*

Introduction

The bluespotted maskray genus *Neotrygon* Castelnau 1873 is widely distributed in the Indo-West Pacific (Last et al. 2016a). The genus is distinguished from other genera in the family Dasyatidae by having the pattern of a dark band in the interorbital region, a black-and-white lateral stripe on the posterior half of the tail, and two papillae in the mouth (Last et al. 2016a). There are currently 16 valid species in the genus (Pavan-Kumar et al. 2018), although the presence of undescribed species has been suggested; the bluespotted maskray from Japan is one of them (Puckridge et al. 2013; Borsa 2017; Borsa et al. 2017, 2019). The Japanese bluespotted maskray was misidentified as *Neotrygon kuhlii* (Müller and Henle 1841) or *Neotrygon orientalis* Last, White and Séret 2016 (Aonuma and Yoshino 2000; Yamaguchi et al. 2013) but is described here as a new species based on morphological and molecular characters.

Materials and methods

Characteristics of the disc follow standards used in Manjaji (2004) and Manjaji-Matsumoto and Last (2006). Morphometric methods in this study, including tail fold measurements, follow Last and White (2008). Institutional codes follow Sabaj (2020). Curatorial procedures for the collected specimens followed Motomura and Ishikawa (2013).

For DNA barcoding, total DNA was extracted from muscle tissue preserved in 99.5% ethanol using the Wizard Genomic DNA Purification Kit (Promega Inc.), following the manufacturer's protocols. The partial sequences of the

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Cytochrome Oxidase subunit I (COI) gene were amplified with a primer cocktail comprising two primers (FishF1, 5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′; FishR1, 5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) (Ward et al. 2005). PCR was conducted in a 25 µL reaction volume containing 7.5 µL of GoTaq Green Master Mix (Promega Inc.), 0.25 µM of each forward and reverse primer, and 1.0 µM of template DNA. The PCR proceeded for 30 cycles, with denaturation at 94℃ for 30 s, annealing at 46℃ for 30 s, and extension at 65℃ for 40 s, with a final extension at 65℃ for 10 min. The PCR products were visualized on 1.2% agarose gels. Sequencing of the samples was performed at Dragon Genomics Center, Takara Bio Inc., Otsu, Japan. Twenty specimens were sequenced in this study and their accession numbers are as follows: KAUM–I. 54079 (accession number: LC782989), 54097 (LC782990), 54957 (LC782992), 54958 (LC782987), 63946 (LC782988), 66176 (LC782985), 87579 (LC782986), 87599 (LC782984), 96575 (LC782983), 101819 (LC782982), 102564 (LC782981), 116618 (LC782980), 120466 (LC782979), 120718 (LC782978), 121010 (LC782977), 145752 (LC782976), 166456 (LC782975), 167810 (LC782974), 170608 (LC782973), NSMT-P 95314 (LC782991). The sequence data generated in this study has been deposited at the DNA Data Bank of Japan (DDBJ). The analysis included a comparison with the following sequences of *Neotrygon*: *Neotrygon annotata* (Last 1987) ($n=3$; accession number: FOAN436-11, FOAN437-11, FOAN438-11), *Neotrygon australiae* Last, White and Séret 2016 ($n=22$; FOA208-04, FOAH738-08, FOAH739-08, FOAI053-08, FOAI054-08, FOAI292-08, FOAI942-08, FOAI943-08, FOAK385-10, FOAL1099-10, FOAM149-10, FOAM150-10, FOAM151-10, FOAN549-11, FOAO1116-18, FOAO1161-18, FOAO1162-18, FOAO1163-18, FOAO1164-18, FOAO1165-18, FOAO1166-18, FOAO1178-18), *Neotrygon bobwardi* Borsa, Arlyza, Hoareau and Shen 2017 ($n=1$; ANGBF48051-19), *Neotrygon caeruleopunctata* Last, White and Séret 2016 ($n=8$; FOAE360-06, FOAE361-06, FOAE362-06, FOAE368-06, FOAE370-06, FOAE371-06, FOAH820-08, FOAK400-10), *Neotrygon indica* Pavan-Kumar, Kumar, Pitale, Shen and Borsa 2018 ($n=15$; DUMBF079-23, DUMBF083-23, DUMBF087-23, DUMBF095-23, GBGC18222-19, GBGC18229-19, GBMNB5972-20, GBMNB5973-20, GBMNB5974-20, GBMNB5975-20, GBMNB5976-20, GBMN73126-21, GBMN73127-21, GBMN73128-21, SAU041-18), *Neotrygon leylandi* (Last 1987) ($n=3$; FOAD400-05, FOAO1170-18, FOAO1173-18), *Neotrygon malaccensis* Borsa, Arlyza, Hoareau and Shen 2017 ($n=1$; ANGBF48038-19), *Neotrygon moluccensis* Borsa, Arlyze, Hoareau and Shen 2017 ($n=1$; ANGBF48131-19), *Neotrygon ningalooensis* Last, White and Puckridge 2010 ($n=8$; FOAI297-09, FOAI298-09, FOAI299-09, FOAI300-09, FOAI305-09, FOAI308-09, FOAI309-09, FOAI327-09), *N. orientalis* Last, White and Séret 2016 ($n=2$; JTFR145-20, JTFR146-20), *Neotrygon picta* Last and White 2008 ($n=20$; ANGBF48181-19, FOA209-04, FOA210-04, FOA211-04, FOA212-04, FOA213-04, FOAH740-08, FOAH741-08, FOAH742-08, FOAH743-08, FOAH744-08, FOAH745-08, FOAH746-08, FOAH747-08, FOAH749-08, FOAH750-08, FOAL1109-10, FOAL1110-10, JTFR04817, JTFR117-20), *Neotrygon trigonoides* (Castelnau 1873) ($n=11$; FOAK382-10, FOAK383-10, FOAK384-10, FOAK386-10, FOAK387-10, FOAK388-10, FOAK401-10, FOAK402-10, FOAK403-10, FOAK404-10, LIFS993-08), *Neotrygon varidens* (Garman 1885) ($n=1$; FOAE373-06), *Neotrygon westpapuensis* Borsa, Arlyza, Hoareau and Shen 2017 ($n=3$; ANGBF48142-19, SOPNG124-18, SOPNG125-18), and Ryukyu maskray (Borsa et al. 2016) ($n=1$; GBGC10609-13) downloaded from Barcode of Life date System (BOLD) and *N. kuhlii* (Müller and Henle 1841) ($n=1$; GT 8223) downloaded from GenBank.

The sequences generated in this study were aligned using Clustal W (Thompson et al. 1994) along with data already published in BOLD and GenBank databases. From the aligned sequences (576 base pair), the best evolutionary model was determined using MEGA X software (Kumar et al. 2018), with the T92 (Tamura 1992) model with Gamma distribution (T92+G) being selected. A maximum likelihood (ML) tree was constructed in MEGA X software, and branch support was measured through nonparametric bootstrapping with 1,000 replications (Felsenstein 1981).

Neotrygon yakkoei sp. nov.

[New English name: Japanese Bluespotted Maskray; standard Japanese name: Yakko-ei]
(Figs. 1, 2, 3, 4, 5, 6, 7; Table 1)

Trygon kuhlii (not of Müller and Henle 1841): Müller and Henle 1841 (in part): 164, pl. 51 (Nagasaki, Japan)

Dasyatis kuhlii (not of Müller and Henle 1841): Jordan et al. 1913: 29 (coasts of Japan and southward); Masuda et al. 1975: 12B (southern Japan); Aonuma and Yoshino 1993: 147 (Hokkaido and south, Japan); Nishida 1997: 58, 59 (Izu Peninsula, Ogasawara Islands, Iriomote-jima Island, Japan); Aonuma and Yoshino 2000: 181 (Hokkaido and south, Japan); Yagishita et al. 2009: table 1, fig. 2 (Ishigaki-jima Island, Yaeyama Islands, Japan); Motomura et al. 2010: 69, fig. 4 (Yaku-shima Island, Japan)

Neotrygon kuhlii Clade 4: Puckridge et al. 2013: figs. 2, 3 (Ishigaki-jima Island, Japan)

Neotrygon kuhlii clade IV: Arlyza et al. 2013: fig. 2 (Ishigaki-jima Island, Japan)

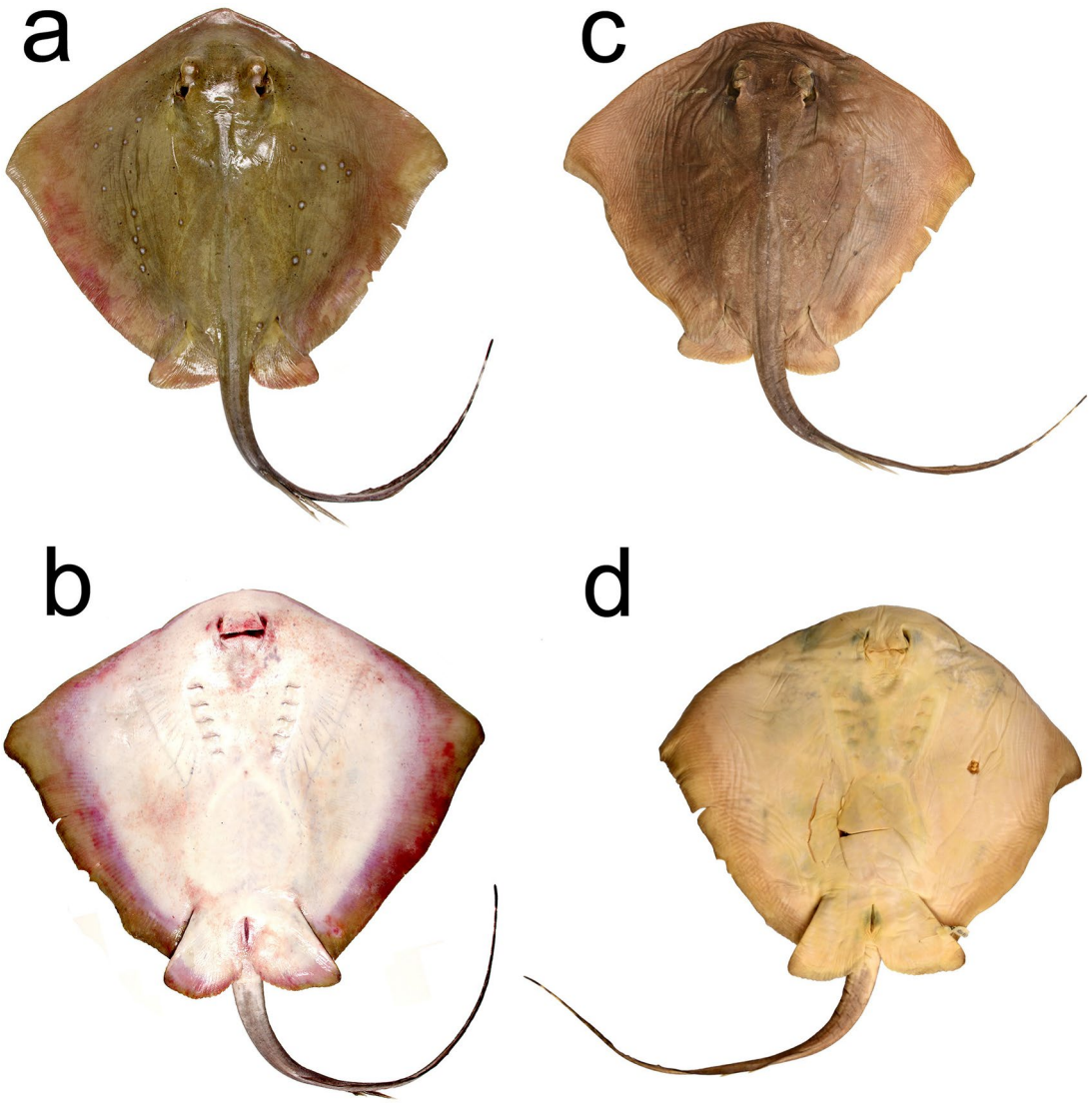

Fig. 1 Holotype of *Neotrygon yakkoei* sp. nov. (KAUM–I. 54097, 333.9 mm DW, Tanega-shima Island, Kagoshima, Japan). **a** Dorsal view of fresh specimen; **b** ventral view of fresh specimen; **c** dorsal view of preserved specimen; **d** ventral view of preserved specimen

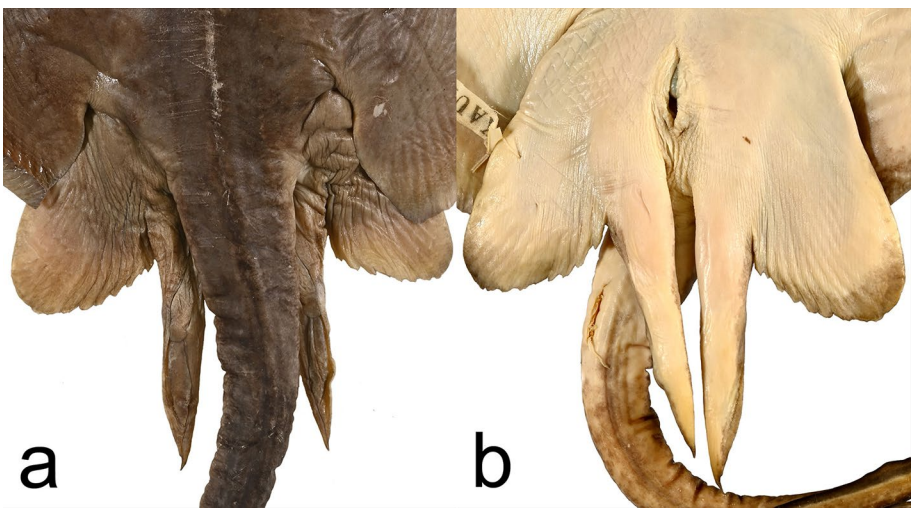

Fig. 2 Clasper of *Neotrygon yakkoei* sp. nov. (paratype: KAUM–I. 167810, 394.8 mm DW, Taira-jima Island, Kagoshima, Japan), showing preserved conditions: **a** dorsal view; **b** ventral view

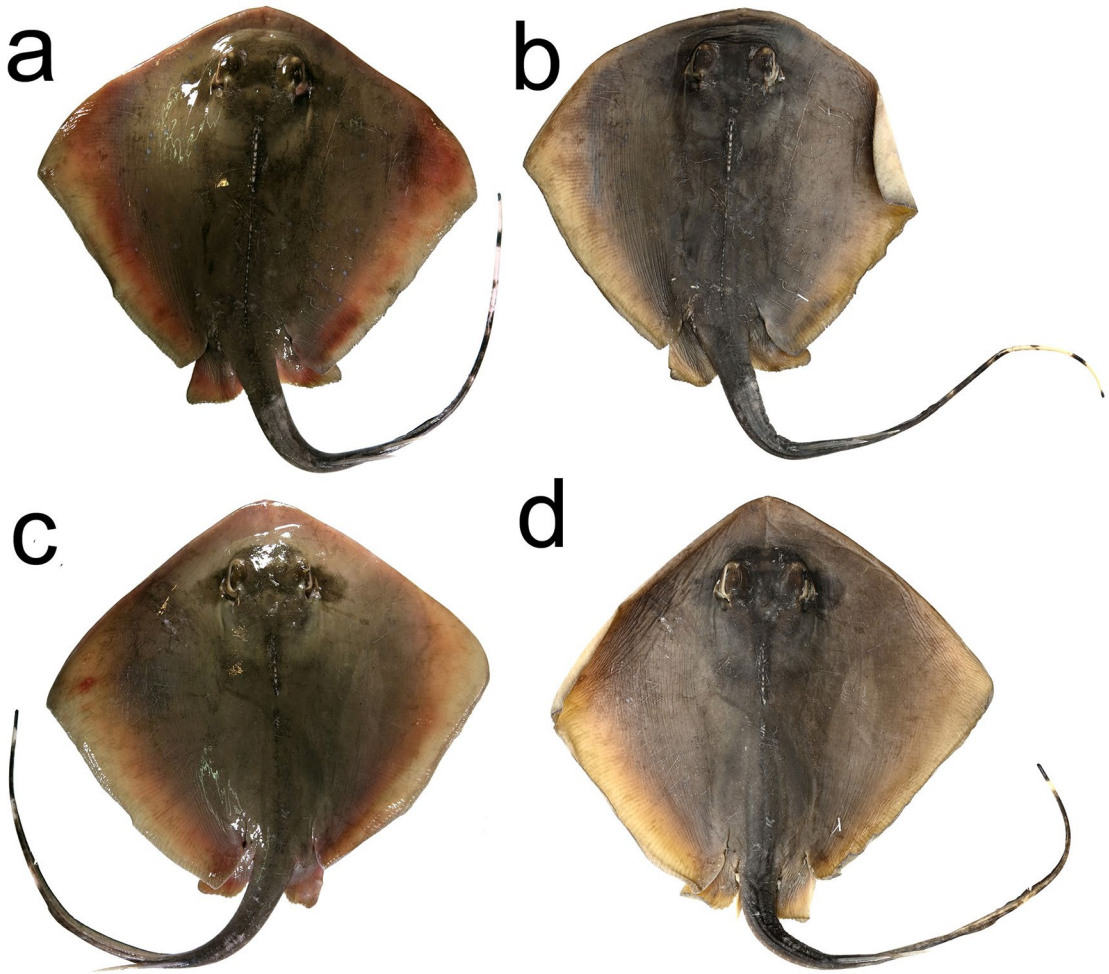

Fig. 3 Specimens of *Neotrygon varidens* from Taiwan [**a**, **b** NMMBP-37314, female, 262.1 mm DW (with some spots); **c**, **d** NMMBP-37321, male, 261.0 mm DW (without spots)], showing fresh (**a**, **c**) and preserved (**b**, **d**) conditions

Neotrygon kuhlii (not of Müller and Henle 1841): Yamaguchi et al. 2013: 224 (coasts of Pacific and Japan Sea of Hokkaido, Hachijo-jima Island, Ogasawara Islands, coast of Pacific from Sagami Bay to southern Kyushu, Wakasa Bay, East China Sea, Ryukyu Islands, Japan); Kato 2014: 23 (Hachijo-jima Island, Izu Islands, Tokyo, Japan); Ikeda and Nakabo 2015: 30, pl. 29-6 (southern Japan); Hata 2017: 23 (Kagoshima Bay, Kagoshima, Japan)

Neotrygon orientalis (not of Last et al. 2016b): Araki 2019: 19 (Amami-oshima Island, Amami Islands, Kagoshima, Japan); Murase et al. 2021: 70, pl. 23 (Kadokawa Bay, Miyazaki, Japan); Bandai and Wada 2022: 19 (Kasasa, Kagoshima, Japan); Motomura 2023: 7, 8 (Tanega-shima Island, Osumi Islands, Kagoshima, Japan)

Holotype. KAUM–I. 54097, female, 333.9 mm disc width (DW), off Kumano, Nakatane, Tanega-shima Island, Osumi Islands, Kagoshima, Japan, 30°28′13″N, 130°58′32″E, 25 m, 14 Apr. 2013, M. Takayama.

Paratypes. 34 specimens (146.4–425.2 mm DW). **Izu Islands: (1 specimen):** KAUM–I. 170608, female, 364.9 mm DW, Mikura-jima Island, Tokyo, Japan, 50–60 m, 24 July 2022. **Ogasawara Islands (1):** NSMT-P 95314, female, 303.3 mm DW, Ougiura, Chichi-jima Island, Tokyo, Japan, 4 Sept. 2009, K. Kuriiwa. **Kyushu (7):** KAUM–I. 140890, male, 291.8 mm DW, Kadogawa Bay, Kadogawa, Higashiusuki, Miyazaki, Japan, 32°28′37″N, 131°39′59″E, 8 m, 24 July 2019, M. Wada; KAUM–I. 14745, female, 216.3 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 4 Mar. 2009, M. Meguro and M. Yamashita; KAUM–I. 28018, male, 209.6 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 31 Mar. 2010, Orita Fishery; KAUM–I. 28772, male, 337.5 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 28 Apr. 2010, Orita Fishery; KAUM–I. 38578, female, 366.5 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 1 June

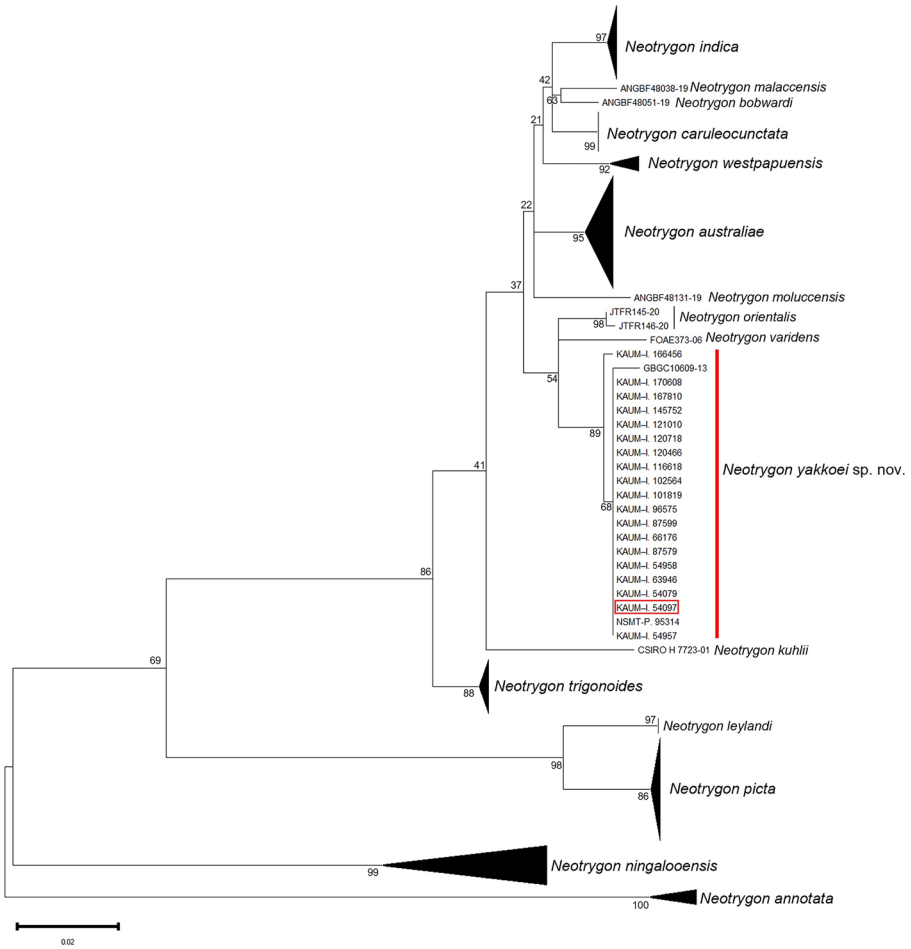

Fig. 4 Maximum likelihood tree based on COI gene sequences (576 bp) of species of *Neotrygon*. Evolutionary distance calculated using T92 (Tamura 1992) with Gamma distribution (T92+G) model. Numbers at nodes indicate bootstrap probabilities (%) of 1,000 resampling. Red frame indicates holotype of *Neotrygon yakkoei* sp. nov.

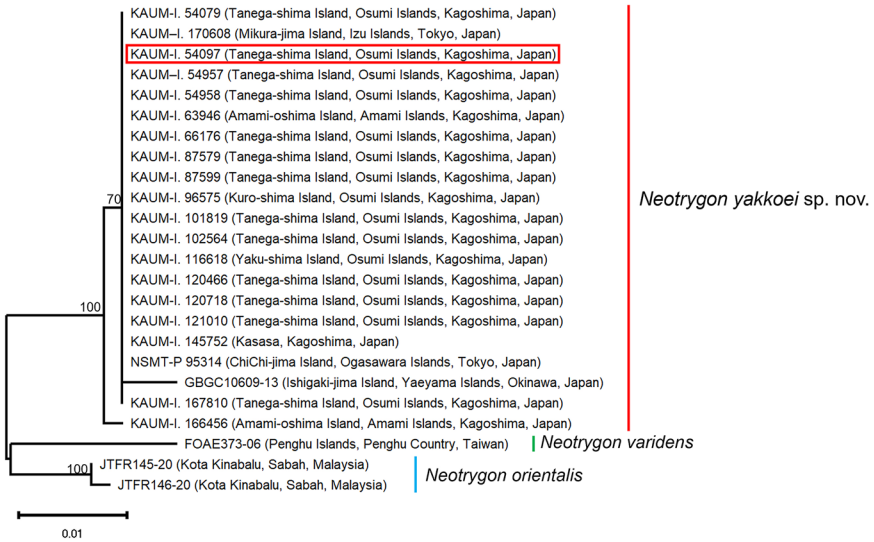

Fig. 5 Maximum likelihood tree based on COI gene sequences (603 bp) of *Neotrygon yakkoei* sp. nov., *N. orientalis* and *N. varidens*. Evolutionary distance calculated using T92 (Tamura 1992) with Gamma distribution (T92+G) model. Numbers at nodes indicate bootstrap probabilities (%) of 1,000 resampling. Red frame indicates holotype of *Neotrygon yakkoei* sp. nov.

2011, M. Megro; KAUM–I. 145752, female, 290.0 mm DW, east of Sakinoyama, Kataura, Kasasa, Minami-satsuma, Kagoshima, Japan, 31°25′44″N, 130°11′49″E, 27 m, 2 July 2020, M. Ito; KAUM–I. 167928, female, 384.7 mm DW, east of Sakinoyama, Kataura, Kasasa, Minami-satsuma, Kagoshima, Japan, 31°25′44″N, 130°11′49″E, 27 m, 26 Apr. 2022, M. Ito. **Osumi Islands (17):** KAUM–I. 186741, female, 348.6 mm DW, off Take-shima Port, Take-shima

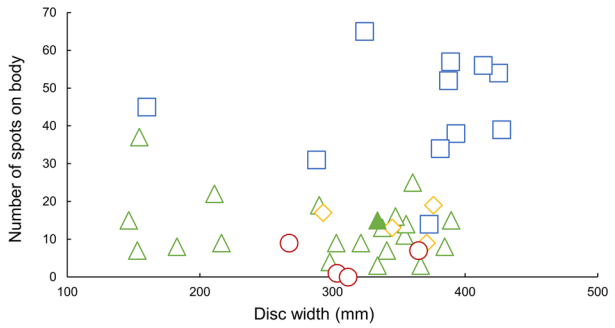

Fig. 6 The frequency distribution of spots on dorsal disc between difference-collection sites of *Neotrygon yakkoei* sp. nov.: Izu Islands and Ogasawara Islands (*open circles*); Kyushu and Osumi Islands (*open triangles*), holotype (*closed triangle*); Amami Islands (*open diamonds*); and Okinawa Islands and Yaeyama Islands (*open squares*)

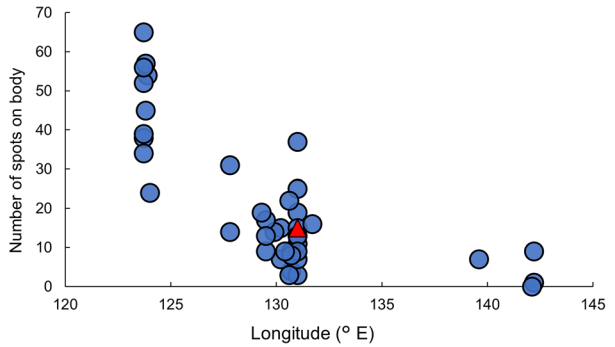

Fig. 7 The relationship between spots and longitude. Red closed triangle indicates holotype of *Neotrygon yakkoei* sp. nov. (15 spots)

Island, Kagoshima, Japan, 30°49′15″N, 130°24′45″E, 10 m, 3 July 2023, T. Yoshida; KAUM–I. 96575, female, 152.8 mm DW, Katatomari Port, Kuro-shima Island, Kagoshima, Japan, 30°49′50″N, 129°54′26″E, 10 m, 21 Nov. 2016, K. Koeda; KAUM–I. 120718, male, 182.7 mm DW, off Osakibana, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°47′N, 131°00′E, 15 m, 15 Sept. 2018, FV *An'ei-maru*; KAUM–I. 54079, male, 321.4 mm DW, off Osaki, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°46′N, 131°00′E, 13 Apr. 2013, M. Takayama; KAUM–I. 121010, male, 146.4 mm DW, vicinity of Nishinoomote North Light House, Nishinoomote Port, Nishimachi, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°44′37″N, 130°58′56″E, 25 m, 3 Oct. 2018, FV *An'ei-maru*; KAUM–I. 87599, female, 302.9 mm DW, KAUM–I. 87600, male, 350.3 mm DW, off Shimoishidera, Shimonishi, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°42′N, 130°57′E, 35 m, 16 May 2016, FV *An'ei-maru*; KAUM–I. 102564, male, 211.0 mm DW, off Shimoishidera, Shimonishi, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°42′N,

Table 1 Morphometric data for the holotype of *Neotrygon yakkoei* sp. nov. (KAUM–I. 54097) and the ranges and means for the measured paratypes. Measurements expressed as a percentage of disc width

	Holotype	Paratypes		
		Min.	Max.	Mean
Disc width (mm)	333.9	146.4	425.2	306.3
Total length	176.4	167.7	198.3	182.1
Disc length	80.4	78.3	90.9	84.3
Snout to pectoral-fin insertion	69.4	67.4	79.5	73.5
Disc thickness	14.2	12.0	16.6	14.1
Snout (preorbital) length	13.5	12.4	17.1	14.7
Snout (preorbital) horizontal length	11.9	9.8	15.1	12.9
Pelvic-fin (embedded) length	17.6	15.0	20.4	18.1
Width across pelvic-fin base	21.0	16.8	23.1	20.0
Greatest width across pelvic fins	39.0	19.8	48.4	35.3
Cloaca origin to tail tip	108.1	97.7	129.7	111.4
Tail width at axil of pelvic fins	7.7	6.5	11.0	8.0
Tail height at axil of pelvic fins	5.3	4.2	6.3	5.0
Pectoral-fin insertion to sting origin	36.8	33.7	50.3	40.0
Cloaca origin to sting	38.9	31.3	49.1	41.0
Tail width at base of sting	2.7	1.9	4.5	2.8
Tail height at base of sting	2.6	1.4	3.8	2.7
Sting 1 length	12.9	4.4	16.6	12.3
Sting 2 length	14.3	10.2	21.8	15.2
Sting 3 length	–	17.2	17.2	17.2
Snout preoral (to lower jaw) length	14.0	12.3	17.0	14.5
Mouth width	8.2	6.1	9.3	7.7
Distance between nostrils	6.9	6.3	8.3	7.3
Interorbital width	7.7	6.2	8.9	7.7
Inter-eye width	17.7	14.0	19.1	16.7
Snout to maximum width	36.6	33.5	43.2	38.4
Eye length	4.9	4.1	7.0	5.3
Orbit diameter	6.8	5.7	9.1	7.3
Spiracle length	7.1	6.1	9.5	7.3
Interspiracular width	14.0	13.5	21.3	15.0
Orbit and spiracle length	9.9	9.2	17.5	10.7
Nostril length	5.0	3.1	5.7	4.5
Snout pre-nasal length	9.0	7.6	12.0	9.8
Nasal curtain length	5.5	4.6	6.8	5.6
Nasal curtain width	8.8	7.8	10.9	9.3
Orbit to pectoral-fin insertion	49.8	47.2	76.1	53.1
Snout to origin of cloaca	68.0	66.1	84.1	71.5
Width 1st gill slit	3.1	2.0	3.5	2.9
Width 3rd gill slit	3.8	2.9	4.0	3.5
Width 5th gill slit	2.3	1.8	3.1	2.5
Head length	36.7	35.9	42.0	39.0
Distance between 1st gill slits	16.2	15.2	18.8	17.1
Distance between 5th gill slits	9.4	8.3	11.8	9.6
Cloaca length	6.7	4.8	8.9	6.6
Clasper postcloacal length	–	9.1	27.6	17.5
Clasper length from pelvic axil	–	12.3	18.2	15.9

130°57′E, 23 m, 28 Apr. 2017, FV *An'ei-maru*; KAUM–I. 66176, female, 389.5 mm DW, off Sumiyoshi, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°39′N, 130°54′E, 17–18 m, 17 Sept. 2014, K. Eguchi and T. Inaba; KAUM–I. 54957, male, 298.0 mm DW, KAUM–I. 54958, male, 347.5 mm DW, KAUM–I. 55347, female, 346.5 mm DW, off Hamathuwaki, Hoshihara, Nakatane, Tanega-shima Island, Kagoshima, Japan, 30°33′N, 130°55′E, 18 June 2013, M. Takayama; KAUM–I. 101819, female, 354.4 mm DW, KAUM–I. 101820, female, 355.6 mm DW, Minamitane, Kumage, Tanega-shima Island, Kagoshima, Japan, 30°27′N, 130°58′E, 17 May 2017, M. Yamada; KAUM–I. 87579, female, 360.5 mm DW, off Tanega-shima Island, Kagoshima, Japan, Apr. 2016, M. Takayama; KAUM–I. 120466, female, 154.3 mm DW, off Tanega-shima Island, Kagoshima, Japan, Sept. 2018, M. Takayama; KAUM–I. 116618, male, 340.9 mm DW, mouth of Miyanoura River, Miyanoura, Yaku-shima Island, Kagoshima, Japan, 30°25′33″N, 130°34′28″E, 2 m, 12 June 2018, J. Nakamura et al. **Tokara Islands (1):** KAUM–I. 167810, male, 384.8 mm DW, Taira-jima Island, Kagoshima, Japan, 28 Aug. 2021, K. Shirasaka. **Amami Islands (2):** KAUM–I. 166456, male, 293.8 mm DW, Naze-nagahama, Amami-oshima Island, Kagoshima, Japan, 28°23′N, 129°29′E, 13 Feb. 2022, S. Agarie; KAUM–I. 63946, male, 376.2 mm DW, Shinokawa Bay, Shinokawa, Setouchi, Amami-oshima Island, Kagoshima, Japan, 28°13′N, 129°17′E, 60 m, 27 June 2014, M. Nakae. **Okinawa Islands (2):** URM-P 2948, male, 372.8 mm DW, Chinen, Okinawa-jima Island, Okinawa, Japan, 18 May 1982; URM-P 17227, female, 287.9 mm DW, Chinen, Okinawa-jima Island, Okinawa, Japan, 16 Apr. 1986. **Yaeyama Island (3):** URM-P 3835, male, 159.9 mm DW, Sumiyoshi, Iriomote-jima Island, Okinawa, Japan, 27 Aug. 1981; KPM-NI 30514, female, 425.2 mm DW, Iriomote-jima Island, Okinawa, Japan; FRMN 13945, female, Funauki Bay, Iriomote-jima Island, Okinawa, Japan, 13 June 1995, S. Kimura et al.

Non-type specimens (identification confirmed, but meristic and morphometric data not taken). 26 specimens (102.1–427.5 mm DW). **Ogasawara Islands (3):** HUMZ 53476, male, 267.2 mm DW, Futami Port, Chichijima Island, Tokyo, Japan, 14 June 1976, 5 m, K. Nakaya; NSMT-P 95343, female, 114.0 mm DW, Ougiura, Chichijima Island, Tokyo, Japan, 4 Sept. 2009, K. Kuriiwa; FRLM 28476, female, 311.8 mm DW, Haha-jima Island, Tokyo, Japan, 22 June 2001, T/V *Seisui-maru*, Mie University. **Kyushu (3):** KAUM–I. 12687, female, 263.1 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 10 Dec. 2008, M. Megro; KAUM–I. 22539, male, 261.7 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 11 Nov. 2009, Orita Fishery; KAUM–I. 28773, female, 330.5 mm DW, off Chiringa-shima Island, Kagoshima, Japan, 31°16′38″N, 130°40′18″E, 25 m, 1 June 2011, M. Megro. **Osumi Islands (8):** KAUM–I. 54078, male, 324.9 mm DW, off Osaki, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°46′N, 131°00′E, 13 Apr. 2013, M. Takayama; KAUM–I. 121009, female, 173.9 mm DW, vicinity of Nishinoomote North Light House, Nishinoomote Port, Nishimachi, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°44′37″N, 130°58′56″E, 25 m, 3 Oct. 2018, FV *An'ei-maru*; KAUM–I. 88217, female, 194.1 mm DW, off Shimoishidera, Shimonishi, Nishinoomote, Tanega-shima Island, Kagoshima, Japan, 30°42′N, 130°57′E, 35 m, 21 May 2016, FV *An'ei-maru*; KAUM–I. 54956, male, 292.0 mm DW, KAUM–I. 55346, female, 371.2 mm DW, off Hamathuwaki, Hoshihara, Nakatane, Tanega-shima Island, Kagoshima, Japan, 30°33′N, 130°55′E, 18 June 2013, M. Takayama; KAUM–I. 54098, female, 298.8 mm DW, Off Kumano, Nakatane, Tanegashima Island, Kagoshima, Japan, 30°28′13″N, 130°58′32″E, 25 m, 14 Apr. 2013, M. Takayama; KAUM–I. 93896, male, 325.0 mm DW, Hommura Port, Yakushima, Kumage-gun, Kuchierabu-jima Island, Osumi Islands, Kagoshima, Japan, 30° 27′41″N, 130°11′29″E, 10 m, 11 Oct. 2016, R. Sakanoue; KAUM–I. 169667, male, 365.0 mm DW, Minamitane, Kumage, Tanega-shima Island, Osumi Islands, Kagoshima, Japan, 30°27′N, 130°58′E, 22 m, 12 May 2022, M. Yamada. **Amami Islands (3):** NSMT-P 124874, male, 371.0 mm DW, NSMT-P 124875, male, 345.0 mm DW, Amami-oshima Island, Kagoshima, Japan, 7 Oct. 2015, S. Yokoyama; NSMT-P 24000, female, 102.1 mm DW, Saneku, Kakeroma-jima Island, Kagoshima, Japan, 7 Oct. 2015, S. Yokoyama. **Okinawa Islands (2):** URM-P 1344, male, 377.2 mm DW, Okinawa Island, Okinawa, Japan, 1960s, Shiro Shinohara; HUMZ 111228, male, 383.7 mm DW, Kuroshima Island, Okinawa, Japan, 10 Feb. 1986. **Yaeyama Islands (7):** NSMT-P 121200, male, 389.0 mm DW, Tsukigahama Beach, Iriomote-jima Island, Okinawa, Japan, 27 Mar. 2011, Y. Imoto; FRLM 13946, male, 136.3 mm DW, Funauki Bay, Iriomote-jima islamd, Okinawa, Japan, 13 June 1995, S. Kimura et al.; FRLM 13953, female, 413.6 mm DW, Funauki Bay, Iriomote-jima Island, Okinawa, Japan, 35 m, 13 June 1995, Y. Iwatsuki; FRLM 22250, female, 427.5 mm DW, Funauki Bay, Iriomote-jima Island, Okinawa, Japan, 24°34′38″N–24°33′05″N, 123°46′26″E–123°44′29″E, 14 May 1998, M. Okada; FRLM 24948, female, 393.1 mm DW, Funauki Bay, Iriomote-jima Island, Okinawa, Japan, 24°34′38″N–24°33′05″N, 123°46′26″E–123°44′29″E, 15 May 2000, S. Kimura et al.; FRLM 48850, male, 381.2 mm DW, Funauki Bay, Iriomote-jima Island, Okinawa, Japan, 7 July 2014, R. Kawamura; FRLM 53049, male, 324.2 mm DW, Funauki Bay, Iriomote-jima Island, Okinawa, Japan, 2 July 2016, S. Yamamoto.

Diagnosis. A species of *Neotrygon* with the following combination of characters: greenish brown body in fresh

with single dark mask-like marking between eyes (often indistinct); fewer spots (mean 20.4); small pale blue spots (in fresh) surrounded by dark brown outer rings [1.2–3.4% DW (2.6% DW)]; longer head length (35.9–43.4% DW; mean 39.1% DW).

Description. Counts and measurements are shown in Table 1. Disc rhombic; pectoral margin straight to slightly curved, convex anteriorly; broader than long, width 1.24 times length in holotype (1.10–1.28 in paratypes); axis of greatest width of disc well forward on disc, slightly forward of scapular region, its distance from snout tip 1.90 (1.69–2.23) times in distance from tip of snout to pectoral-fin insertion; body relatively thick, thickness 7.0 (6.0–8.3) times in disc width, raised just above cranium; apex of disc broadly rounded, narrowly or abruptly angular, pectoral angle 89° (85–91°); posterior margin straight to slightly curved, weakly undulate; free rear tip narrowly angular. Pelvic fins subtriangular, anterior margin nearly straight; tip rounded; posterior margin rounded, concave and convex; inner margin fused with tail, not independent; rather small; 0.84 (0.79–1.09) times in width across fin bases; claspers of mature males relatively narrow, tapering toward the tip, pointed apically; center of dorsal profile depressed, ventral profile not depressed and smooth.

Tail relatively broad at base, width 1.46 (1.21–2.10) times in depth, tapering toward tip of tail and abruptly behind base of sting(s); dorsal and ventral midlines of posterior half of tail with skin folds; dorsal-skin fold semi-elliptical, height 0.7% (0.3–1.3%) DW, length 9.1% (5.1–10.8%) DW; ventral skin fold rounded trapezoidal, height 1.6% (0.8–1.6%) DW, length 51.6% (44.8–79.7%) DW, very long, 5.69 (2.76–11.30) times dorsal length, slightly high, 2.41 (1.11–4.62) times dorsal height. Tail with 2 (1–3) caudal stings (broken or absent in some).

Snout thick (thicker in mature paratypes, thinner in immature paratypes), short, relatively broad angle 122.7° (118.6–132.6°); apex slightly pointed (slightly rounded in some immature paratypes); when viewed horizontally, snout slightly upward; preoral snout length 1.69 (1.48–2.62) times mouth width, 2.03 (1.47–2.35) times internarial distance, 0.86 (0.65–1.02) times distance between first gill slits; preorbital snout short, direct length 1.77 (1.51–2.32) times interorbital length; snout to maximum disc width 2.73 (2.32–2.98) in DW; interorbital space narrow and flat; eyes large, dorsolateral of head, protruding (slightly protruding in some small paratypes); orbit diameter 0.97 (0.90–1.26) in spiracle length; eye length 1.43 (1.17–1.52) in spiracle length; inter-eye width 3.59 (2.46–3.63) times eye length. Spiracles large, crescent-shaped, with a dorsal opening, situated just after eyes. Nostril slit-like, elongated, separated from each other; outer margin slightly thicker than inner margin; nasal fold in nostril internarial space 1.31 (1.02–1.81) in pre-nasal length, 1.38 (1.37–2.42) times nostril length. Nasal curtain relatively short, reaching mandible, skirt-shaped, width 1.62 (1.39–2.16) times length; posterior margin with folds, concave in center; posterior margins except middle slightly arched.

Mouth small, not protruding forward, gently arched, convex above; a pair of papillae on each side (two in total) in mouth; rows of teeth in upper jaw less than 33, lower jaw less than 39; the number of upper and lower teeth differed from 3–6; upper jaw with rounded teeth (adult male paratypes have sharply pointed teeth); lower jaw with rounded teeth in anterior portion of mouth, slightly more pointed toward posterior portion (adult male paratypes have sharply pointed teeth throughout); mandible wrinkled.

Gill openings elongate S-shaped, five pairs, separated from each other; length of first gill slit 1.33 (1.04–1.56) times length of fifth gill slit, 2.64 (1.85–3.29) times in mouth width; distance between first gill slits 2.36 (2.12–2.63) times internarial space, 0.44 (0.40–0.49) times head length (snout to fifth gill slit), 1.73 (1.29–1.97) times distance between fifth gill slit; distance between fifth gill slits 1.37 (1.14–1.79) times internasal distance, 0.26 (0.22–0.31) times ventral head length.

Color. *Live coloration* (based on holotype). Dorsal surface of disc (Fig. 1a) uniformly greenish brown; dermal denticles white; small pale bluish spots on disc with dark brown margin; mask-like marking around eyes darker brown; tiny black spots scattered on disc, except at the margin of disc, and more numerous around eye area than on disc; eyes whitish; tail dark brown, and beyond stings whitish gray; posterior half of tail black and white transverse stripes; stings blackish white; dorsal fold blackish gray. Ventral surface of disc (Fig. 1b) uniformly whitish; margins of pectoral and ventral fins brown; base of tail white, tail behind posterior end of pelvic fin blackish gray; ventral fold long, blackish gray.

In preserved state (based on holotype excepted claspers). Dorsal surface of disc (Fig. 1c) uniformly dark brown; dermal denticles white; small brownish white spots on disc with dark brown margin; some areas of pectoral fin greenish black stripes; mask-like marking around eyes darker brown; tiny black spots around eyes and disc area still visible; eyes white; tail uniformly blackish brown; claspers in mature specimen (Fig. 2a) uniformly dark brown, slightly lighter dark brown ahead; posterior half of tail with brownish black and white transverse stripes; stings blackish white; dorsal fold dark brown. Ventral surface of disc (Fig. 1d) uniformly brownish white; margins of pectoral and ventral fins darker brownish white; base of tail brownish white, tail behind posterior end of pelvic fin blackish brown; mature male claspers (Fig. 2b) uniformly yellowish white with yellowish brown outer margin; ventral fold, darker blackish brown.

Distribution. *Neotrygon yakkoei* sp. nov. is distributed along the Japan Sea coast and Pacific coast of Hokkaido,

Wakasa Bay, the Izu Islands, the Ogasawara Islands, Pacific Ocean from Sagami Bay to the southern coast of Kyushu, the Osumi Islands, the Amami Islands, the Okinawa Islands, and the Yaeyama Islands (Yamaguchi et al. 2013; Murase et al. 2021; this study).

Etymology. The specific name "yakkoei" is derived from the standard Japanese name "yakkoei".

Comparisons. Because of the morphological similarity among *Neotrygon yakkoei* sp. nov., *N. orientalis* and *N. varidens*, the new species has often been considered to be conspecific (Araki 2019; Murase et al. 2021; Bandai and Wada 2022; Motomura 2023).

Compared to *N. orientalis*, *N. yakkoei* sp. nov. has a greenish-brown body (this study: Fig. 1a) [a yellowish brown body in *N. orientalis* (Last et al. 2016b)], an indistinct dark mask-like marking between eyes (this study: Fig. 1a) [a distinct dark mask-like marking between eyes (this study)], small pale blue spots with dark brown margin (this study: Fig. 1a) [medium blue spots with slightly darker blue margin (Last et al. 2016a, b; this study)], a smaller spot diameter 1.2–3.4% DW (mean 2.6% DW) (this study) [diameter 2.5–4.8% DW (4.1% DW) (this study)], fewer spots on the disc and mean 20.4 spots (this study) [40.2 spots (this study)], and relatively longer head length of 35.9–43.4% DW (39.1% DW) (this study) [34.7–38.2% DW (36.1% DW) (this study)]. In addition, *Neotrygon orientalis* has spots on pelvic fins, while absent in *N. yakkoei* (this study).

Neotrygon varidens is very similar to the new species in disc shape. Compared to *N. varidens*, *N. yakkoei* sp. nov. has spots that are visible in both fresh and preserved conditions (Figs. 1, 2), whereas *N. varidens* rarely display spots in fresh, and all spots disappear or become indistinct after preservation (Fig. 3). The coloration of spots in preserved specimen is dark brown in *N. yakkoei* sp. nov., while gray or blackish gray in *N. varidens* (this study).

Also, the maximum disc width of *N. orientalis* and *N. varidens* are 380 mm and 330 mm (Last et al. 2016a, b), respectively, while *N. yakkoei* sp. nov. reaches 427.5 mm (this study). Therefore *N. yakkoei* sp. nov. appears to be larger than *N. orientalis* and *N. varidens* (this study).

The holotype of *Neotrygon yakkoei* sp. nov. (KAUM–I. 54097) showed 1.9%–13.8% base substitutions when compared to other species of the genus *Neotrygon*, except for *Neotrygon vali* Borsa 2017 in a comparison of 576 bp in the partial COI region (Fig. 4), and *N. yakkoei* from Japan showed 0.0%–0.5% base substitutions. Genetic distances between *N. yakkoei* sp. nov. and closely related two species *N. orientalis* and *N. varidens* were 1.9–2.6% and 2.5–3.1%, respectively (Fig. 5). *Neotrygon vali* was described in Borsa (2017) by the genetic character, although the sequence and morphological information were not documented. The validity of *N. vali* is followed Borsa (2017) which regards the Ryukyu maskray (= *N. yakkoei* sp. nov.) and *N. vali* as genetically distinct species, based on the molecular analysis of the partial COI region.

Remarks. The number of stings in the new species varies in growth. Specimens larger than 300 mm DW usually have two stings, except for cases where the stings are absent, likely due to being cut off. Smaller specimens have a single sting. There is an exception with one specimen (KAUM–I. 101819, 354.4 mm DW) which has three stings. However, all specimens larger than 354.4 mm DW have two stings, suggesting that the three stings are not a growth variation, but rather a mutation.

This study reveals that the number of spots in *Neotrygon yakkoei* sp. nov. exhibits geographic variation (Fig. 6). Specimens collected from the west of Japan, such as in the Okinawa and Yaeyama Islands, have numerous spots [Fig. 6: 14–65 spots (mean 42.4 spots)], while specimens from the east of Japan, such as in the Ogasawara Islands, have fewer spots [Fig. 6: 0–9 spots (mean 5.6 spots)]. In other words, the number of spots increases from east to west (Fig. 7).

Figure 1 of *N. kuhlii*, as shown by Last et al. (2016b) (= *Trygon kuhlii* in Müller and Henle 1841), was drawn by the Edo period painter Keiga Kawahara based on a specimen obtained from Nagasaki, Japan (Borsa and Béarez 2016). The species depicted in this figure is not *N. kuhlii*, but *N. yakkoei* sp. nov., based on the collection locality and the spots of the disc (Last et al. 2016b: fig. 1).

Comparative material examined. *Neotrygon orientalis* (6 specimens, 143.4–348.9 mm DW): CSIRO H 7848-01, female, 348.9 mm DW, Singkawang fish market, Kalimantan, Indonesia, 00°55′11″N, 108°58′99″E, 28 July 2007, J. Caira and K. Jensen; CSIRO H 7099-10, female, 143.4 mm DW, Flamboyan market, Pointianak, Kalimantan, Indonesia, 00°02′34″N, 109°20′64″E, 12 July 2008, J. Caira and K. Jensen; CSIRO H 6136-02, male, 237.7 mm DW, CSIRO H 6136-04, male, 228.0 mm DW, Muara Angke fish market, Jakarta, Java, Indonesia, 06°06′S, 106°48′E, 31 Jan. 2003, Indo Oz project and W. White; CSIRO H 7858-01, male, 213.0 mm DW, Muara Kintap, Kalimantan, Indonesia, 03°54′26″N, 115°15′53″E, 30 Nov. 2006; CSIRO H 7849-01, female, 291.3 mm DW, Muara Angke fish market, Jakarta, Java, Indonesia, 06°06′S, 106°48′E, 8 Oct. 2009, Indo Oz project and W. White. *Neotrygon varidens* (12 specimens, 167.0–316.7 mm DW): HUMZ 213854, male, 272.8 mm DW, Tashi, Taiwan, 14 Mar. 2012; KAUM–I. 178500, female, 292.5 mm DW, KAUM–I. 178501, female, 256.3 mm DW, KAUM–I. 178502, female, 190.3 mm DW, KAUM–I. 178503, female, 167.0 mm DW, KAUM–I. 178504, female, 188.4 mm DW, KAUM–I. 178505, female, 201.9 mm DW, KAUM–I. 178506, female, 316.7 mm DW, NMMBP-37314, female, 262.1 mm DW, NMMBP-37321, male, 261.0 mm DW, Taiwan; HUMZ 13930, female, 283.0 mm DW, South China Sea, 04°00′N, 105°49′E, 20

Dec. 1957; FRLM 44070, female, 225.5 mm DW, Johor Strait, Merambong Shoal, Johor, Malaysia, 01°33′N, 103°05′E, 2 Dec. 2012, S. Kimura et al.

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Declarations

Conflicts of interest The authors declare no conflicts of interest.

Ethics approval Not applicable.

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