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Cover: Mauve Stinger *Pelagia noctiluca* by Swaathi Na. Medium used is soft pastels and gelly roll.



First record of *Tanaorhinus viridiluteata* Walker, 1861 (Lepidoptera: Geometridae: Geometrinae) from Mizoram, India

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Abstract: Very little work has been done to document the moth fauna of the Mizoram state in northeast India. An emerald moth collected from three localities in Aizawl District of Mizoram was identified as *Tanaorhinus viridiluteata* Walker, 1861 based on morphological and molecular studies. This species has been described briefly with colour photographs of male and female genitalia. Partial mitochondrial COI gene was amplified from these specimens for molecular analysis. This study represents a first record of the genus *Tanaorhinus* and species *T. viridiluteata* from Mizoram State.

Keywords: COI, genitalia, maximum likelihood, morphology, northeastern India, type locality.

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Author contributions: BL—field survey, curation, morphological & molecular data collection, data analyses, writing original draft; LA—field survey, curation, morphological data collection, data analysis; EL—conceptualization, methodology analysis, morphological & molecular data analyses, writing original draft & review & editing, supervision.

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INTRODUCTION

Mizoram State is situated in the southernmost tip of northeastern India, sandwiched by Myanmar in the east and Bangladesh in the west. Though the area falls within the Indo-Burma biodiversity hotspot (Mittermeier et al. 2004), the flora and fauna of the area are poorly documented. However, recent studies taken up in the area resulted in the description of several species new to science (e.g., Lalronunga et al. 2013; Giri et al. 2019; Kirti et al. 2019; Naumann & Lalmingliani 2019). Hebert et al. (2003) proposed the use of the mitochondrial gene cytochrome c oxidase I (COI) as a reliable marker for accurate species identification, particularly in animals. Though faced with many criticisms and pitfalls (e.g., Tautz et al. 2003; Blaxter 2004), it is a useful tool for the identification of lepidopterans in general (Hajibabaei et al. 2006; Kim et al. 2020) and geometrid moths in particular (Brehm et al. 2016; Kumar et al. 2019).

Geometrinae (commonly known as emerald moths) is the fourth largest subfamily in the family Geometridae, with more than 27,006 valid species-group names, including 23,872 species and 3,123 subspecies worldwide (Rajaei et al. 2022). The genus *Tanaorhinus* Butler, 1879 contains 16 nominal species and five subspecies (Scoble & Hausmann 2007; Orhant 2014; Tautel 2014; Rajaei et al. 2022) all are restricted to Asia (Scoble 1999). However, Ban et al. (2018) revealed that the genus *Geometra* and *Tanaorhinus* are polyphyletic and revived the genus *Loxochila* Butler 1881 to accommodate *G. burmensis*, *G. fragilis*, *G. sinoisaria*, *G. smaragdus*, *T. kina*, and *T. tibeta*. They further speculated *Tanaorhinus* to be a junior synonym of *Geometra*. However, further molecular studies with the inclusion of more taxa are required for formal taxonomic action (Ban et al. 2018). Five species, viz., *T. celebensis* Yazaki, 1995, *T. kina kina* Swinhoe, 1893, *Tanaorhinus kina embrithes* Prout, 1934, *T. rafflessi* (Moore, [1860]), *T. reciprocate reciprocata* (Walker, 1861), and *T. viridiluteata* (Walker, 1861), were recorded from India (Kirti et al. 2019). Walker (1861) described *T. viridiluteata* from Darjeeling in West Bengal State of India. Apart from the type locality, the species was further recorded from Arunachal Pradesh, Assam, and Nagaland states in northeastern India, southern China, Taiwan, and Sundaland (Anonymous 2021; Holloway 2021). The species is characterized by dark green colour with two black cell specks enclosed by a bluish tinge on both sides of the forewing, ante and post medial waved lines closed together with irregular white suffusion on dorsal side of the body. The ventral side of the forewing is heavily suffused with brown and mauve and inner

margin of the hindwing is excised forming heart shaped gap. This species is most similar to *T. rafflesia*, but can be distinguished from it by the presence of broad, uniform, rufous border in the ventral hindwing (vs. narrower and separated from the margin by a yellow zone in *T. rafflesia*) in males; and harpe in male genitalia more spatulated (vs. more acute in *T. rafflesia*). Herein, we report the first distribution records of *T. viridiluteata* from the state Mizoram in northeastern India.

MATERIALS AND METHODS

Surveys were conducted in Mizoram State (see materials examined section under results and discussion for details) using a 160 W mercury vapour bulb on a 4 ft. by 6 ft. white cloth screen with a Honda™ EP1000 portable generator as a power source. Specimens were killed in a killing jar containing petroleum ether, which were then removed and placed on butter paper with their wings folded vertically. Pinning, spreading, and labeling of specimens were done in the laboratory. Specimens were deposited in the Entomological Collections of the Systematics and Toxicology Laboratory, Mizoram University, Mizoram, India (MZUEC). Tissue (three legs each) was collected in a 2 ml centrifuge tube for genomic DNA extraction. The genitalia of each specimen were dissected following Sondhi (2020). Genomic DNA was extracted from the tissue sample using 10 µl of 20 mg/ml of Proteinase K with 56°C overnight treatment following standard Phenol: Chloroform: Isoamyl alcohol method (Sambrook & Russell 2001). We amplified a partial mitochondrial COI gene using the primer pair LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR amplification was carried out in 25 µl aliquots containing 12.5 µl of EmeraldAmp® GT PCR Master Mix (2X) (TaKaRa Bio, Japan), 1 µl of each forward and reverse primer, 2 µl of genomic DNA, and 8.5 µl of molecular grade H₂O using ProFlex™ 3 x 32-well PCR system (Applied Biosystems™, USA). The PCR conditions were as follows: initial denaturation was performed at 95°C for 5 min, followed by 35 or 40 cycles of 30 s at 94°C, 30 s annealing from 42°C to 50°C (Tables S3 and S4), 30 s at 72°C, with a final 5 min extension at 72°C. Amplified PCR products were ran on 1.5% agarose gel, viewed in IG-618GD (iGene Labserve, India) gel documentation system. The purified PCR products were sequenced bidirectionally by Sanger sequencing technology at geneOmbio Technologies Private Limited (Maharashtra, India). The chromatograms and raw sequences were edited using FinchTV 1.4.0 (Geospiza Inc., USA) and the consensus

sequences were checked by BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). The generated sequences (615–618 base pairs) were submitted to GenBank (NCBI) to acquire the accession numbers (MW855164–MW855166). The newly generated sequences were compared with other sequences of *Tanaorhinus* available in GenBank (Supplementary Table 1). Based on the lowest BIC (Bayesian Information Criterion) and AICc

scores (Akaike Information Criterion, corrected), best fit nucleotide substitution model for the present COI dataset was GTR+G+I. A Maximum Likelihood (ML) tree was constructed with 1000 bootstraps in MEGA X (Kumar et al. 2018). The barcode data of *Chlorozancla falcatus* (MG014741) was used as an outgroup in the present phylogenetic analysis. The uncorrected pairwise genetic distances (p-distances) between and within the studied species were estimated by MEGA X (Kumar et al. 2018).

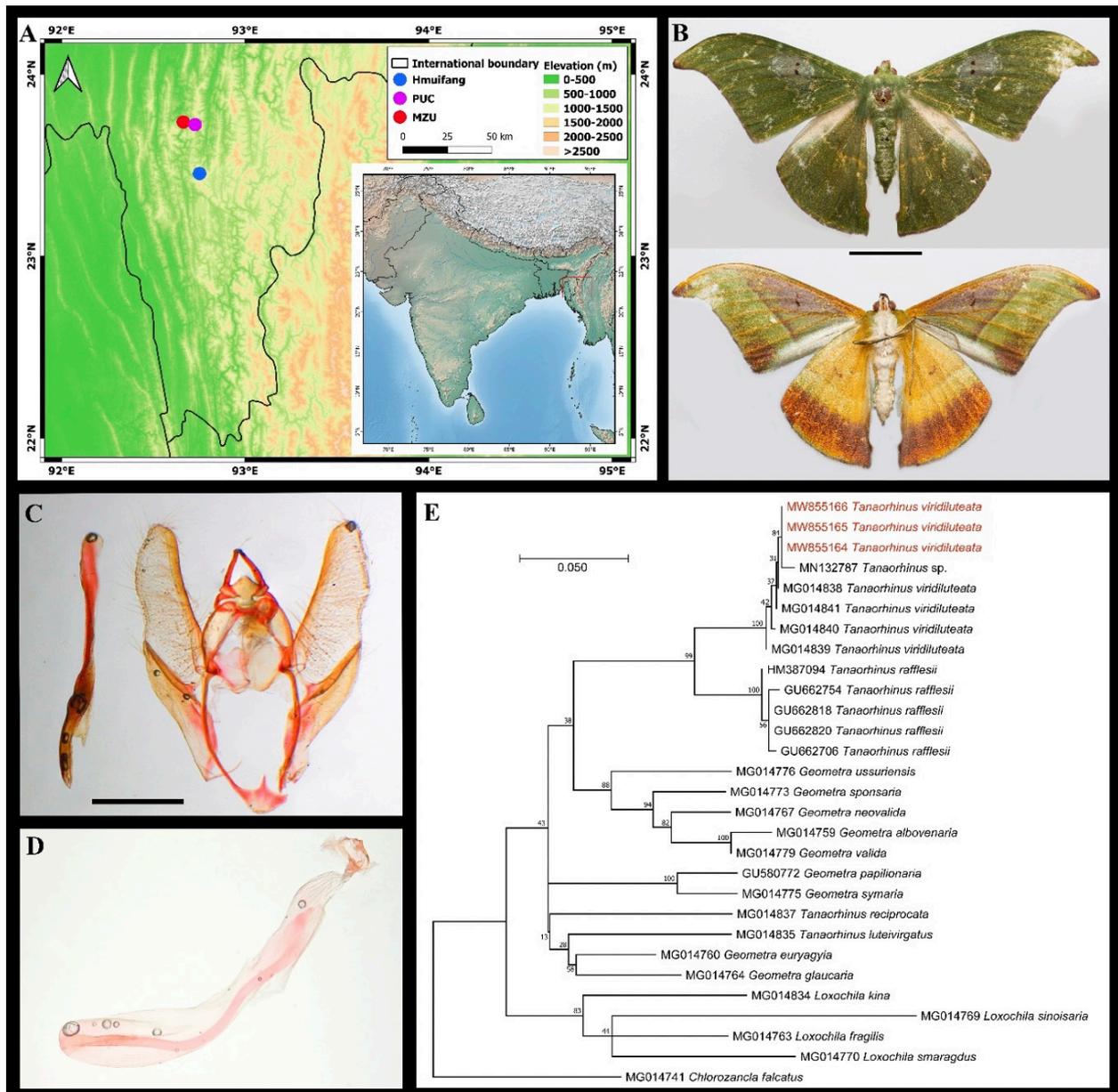


Image 1 . A—Collection localities of *Tanaorhinus viridiluteata* from Mizoram, India | B—Dorsal and ventral aspects of male individual (MZUEC 20210008); scale bar 1 cm | C—Male genitalia (MZUEC 20210004); scale bar 1 mm | D—Female genitalia (MZUEC 20210006), scale bar 1 mm | E—Maximum likelihood (ML) tree of the 29 COI sequence dataset; letters in red indicates the newly generated sequences for the present study. © B, C & D—B. Lalngahpuii.

Material examined

MZUEC 20210001–20210005; 03.xii.2020; Hmuifang community forest reserve, Aizawl District, Mizoram; coll. B. Lalngahpuii & party; (23.0051°N 92.7521°E, elevation 1,480 m). MZUEC 20210006–20210007; 23.x.2020; 01 female (wingspan 62 mm), 01 male (wingspan 65 mm); Mizoram University Campus, Aizawl District, Mizoram; coll. B. Lalngahpuii & party; (23.7370°N 92.6636°E, elevation 790 m). MZUEC 20210008–20210009; 15.xi.2019; 01 female (wingspan 68 mm), 01 male (wingspan 60); Pachhunga University Campus, Aizawl District, Mizoram; coll. B. Lalngahpuii & party; (23.7234°N 92.7307°E, elevation 815 m (Image 1A)).

Diagnosis: Wingspan 60–64 mm in male (four specimens) and 72 mm in female (one specimen). Upperside of forewing dark green in colour with two black cell specks enclosed by a bluish tinge; ante and post medial waved lines closed together with irregular white suffusion on dorsal side of the body; lunulate markings absent beyond the postmedial line; obscure white marks on submarginals. Underside of forewing green with costal area to beyond cell purplish-grey; oblique postmedial line with rufous patches at apex and outer angle; inner margin white. Upperside of hind wing dark green in colour except for costa which is white. Underside of hindwing yellowish with traces of postmedial line; outer area rufous; outer marginal areas yellowish (Image 1B).

The specimens collected from the three localities in Mizoram agreed with the description of *Tanaorhinus viridiluteata* in possessing the following characters: dark green colour with two black cell specks enclosed by a bluish tinge on both sides of the forewing; ante and post medial waved lines closed together with irregular white suffusion on the dorsal side of the body; ventral side of forewings heavily suffused with brown and mauve; presence of a broad, uniform, rufous border in the ventral hindwing in males; slightly spatulated harpe in male genitalia. The maximum likelihood (ML) tree (Image 1E) further revealed that the sample sequences from Mizoram, India, formed a clade with the sequences of *T. viridiluteata* along with an undetermined species of *Tanaorhinus* with an uncorrected genetic distance (p-distance) of only 0.002–0.008. In the ML tree, *T. viridiluteata* and *T. rafflesia* are sister species, which is not surprising as the two species are very similar morphologically. The genetic distance between the two species ranges from 0.053–0.061.

DISCUSSION AND CONCLUSION

As far as Mizoram is concerned, little work has been done to document the moth fauna of the state (Ghosh 2007; Kirti & Singh 2014, 2016; Kirti et al. 2014, 2019; Lalhmingliani et al. 2013, 2014; Lalhmingliani 2015), but recent studies have led to the description of several new species (e.g., Kirti et al. 2019, Naumann & Lalhmingliani 2019). Ghosh (2007) and Kirti et al. (2014, 2019) reported on geometrid moths from Mizoram State but did not mention the genus *Tanaorhinus*. The present study on *Tanaorhinus viridiluteata* from Mizoram represents the first record for this genus and species in the state.

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Supplementary Table 1. Cytochrome Oxidase subunit I (COI) gene sequences used for molecular analysis in this study.

	Species	Voucher code	Collection Locality	GenBank accession number	References
1	<i>Tanaorhinus viridiluteatus</i>	IOZ LEP M 10029	Fujian, China	MG014838	Ban et al. 2018
2	<i>Tanaorhinus viridiluteatus</i>	IOZ LEP M 2325	Hainan, China	MG014839	Ban et al. 2018
3	<i>Tanaorhinus viridiluteatus</i>	IOZ LEP M 8110	Hainan, China	MG014840	Ban et al. 2018
4	<i>Tanaorhinus viridiluteatus</i>	IOZ LEP M 8283	Guangdong, China	MG014841	Ban et al. 2018
5	<i>Tanaorhinus viridiluteatus</i>	MZUEC 20210004	Mizoram, India	MW855164	Present study
6	<i>Tanaorhinus viridiluteatus</i>	MZUEC 20210006	Mizoram, India	MW855165	Present study
7	<i>Tanaorhinus viridiluteatus</i>	MZUEC20210008	Mizoram, India	MW855166	Present study
8	<i>Tanaorhinus rafflesii</i>	RMNH.INS.13846	Kalimantan Timur, Indonesia	HM387094	GenBank
9	<i>Tanaorhinus rafflesii</i>	RMNH.INS.14079	Kalimantan Timur, Indonesia	GU662706	GenBank
10	<i>Tanaorhinus rafflesii</i>	RMNH.INS.13847	Kalimantan Timur, Indonesia	GU662754	GenBank
11	<i>Tanaorhinus rafflesii</i>	RMNH.INS.13845	Kalimantan Timur, Indonesia	GU662818	GenBank
12	<i>Tanaorhinus rafflesii</i>	RMNH.INS.13843	Kalimantan Timur, Indonesia	GU662820	GenBank
13	<i>Tanaorhinus</i> sp.	Lep8581	China	MN132787	Wang et al. 2019
14	<i>Tanaorhinus luteivirgatus</i>	IOZ LEP M 16545	Yunnan, China	MG014835	Ban et al. 2018
15	<i>Tanaorhinus reciprocata</i>	IOZ LEP M 17064	Gansu, China	MG014837	Ban et al. 2018
16	<i>Geometra albovenaria</i>	IOZ LEP M 5523	Shaanxi, China	MG014759	Ban et al. 2018
17	<i>Geometra euryagyia</i>	IOZ LEP M 16429	Shaanxi, China	MG014760	Ban et al. 2018
18	<i>Geometra glaucaria</i>	IOZ LEP M 16501	Beijing, China	MG014764	Ban et al. 2018
19	<i>Geometra neovalida</i>	IOZ LEP M 4763	Shaanxi, China	MG014767	Ban et al. 2018
20	<i>Geometra papilionaria</i>	NS03	Avinurme, Estonia	GU580772	Wahlberg et al. 2010
21	<i>Geometra sponsaria</i>	IOZ LEP M 8581	Liaoning, China	MG014773	Ban et al. 2018
22	<i>Geometra symaria</i>	IOZ LEP M 9287	Hubei, China	MG014775	Ban et al. 2018
23	<i>Geometra ussuriensis</i>	IOZ LEP M 4682	Shaanxi, China	MG014776	Ban et al. 2018
24	<i>Geometra valida</i>	IOZ LEP M 8567	Liaoning, China	MG014779	Ban et al. 2018
25	<i>Loxochila fragilis</i>	IOZ LEP M 9212	Yunnan, China	MG014763	Ban et al. 2018
26	<i>Loxochila kina</i>	IOZ LEP M 17078	Tibet, China	MG014834	Ban et al. 2018
27	<i>Loxochila sinoisaria</i>	IOZ LEP M 9457	Sichuan, China	MG014769	Ban et al. 2018
28	<i>Loxochila smaragdus</i>	IOZ LEP M 16551	Yunnan, China	MG014770	Ban et al. 2018
29	<i>Chlorozancla falcatus</i>	IOZ LEP M 20201	Guangxi, China	MG014741	Ban et al. 2018

Supplementary Table 2. Uncorrected p-distances between Cytochrome Oxidase subunit I (COI) gene sequences used in the study. GenBank accession numbers are listed after the name of the species. Letters in bold indicates the newly generated sequences in this study.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Geometra albovenaria</i> (MG014759)																													
<i>Geometra eurygyia</i> (MG014760)	0.089																												
<i>Geometra fragilis</i> (MG014763)	0.093	0.091																											
<i>Geometra glaucaria</i> (MG014764)	0.078	0.048	0.089																										
<i>Geometra neovalida</i> (MG014767)	0.042	0.070	0.068	0.055																									
<i>Geometra papilionaria</i> (GU580772)	0.091	0.078	0.112	0.074	0.086																								
<i>Geometra sinoisaria</i> (MG014769)	0.118	0.116	0.095	0.106	0.112	0.116																							
<i>Geometra smaragdus</i> (MG014770)	0.131	0.112	0.086	0.110	0.112	0.129	0.105																						
<i>Geometra sponsaria</i> (MG014773)	0.065	0.061	0.089	0.057	0.040	0.089	0.122	0.116																					
<i>Geometra synaria</i> (MG014775)	0.093	0.076	0.110	0.067	0.084	0.044	0.114	0.131	0.082																				
<i>Geometra ussuriensis</i> (MG014776)	0.072	0.068	0.086	0.065	0.049	0.087	0.110	0.118	0.061	0.084																			
<i>Geometra valida</i> (MG014779)	0.023	0.072	0.082	0.057	0.029	0.087	0.114	0.118	0.042	0.082	0.059																		
<i>Tanaorhinus viridiluteata</i> (MG014838)	0.099	0.089	0.112	0.078	0.087	0.103	0.118	0.125	0.089	0.103	0.097	0.087																	
<i>Tanaorhinus</i> sp. (MN132787)	0.097	0.087	0.114	0.080	0.086	0.106	0.120	0.125	0.087	0.106	0.093	0.086	0.008																
<i>Tanaorhinus viridiluteata</i> (MG014841)	0.097	0.087	0.110	0.076	0.086	0.103	0.116	0.124	0.087	0.103	0.095	0.086	0.002	0.006															
<i>Tanaorhinus viridiluteata</i> (MG014840)	0.097	0.091	0.112	0.080	0.086	0.105	0.116	0.127	0.089	0.105	0.091	0.086	0.006	0.006	0.004														

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Tanaorhinus viridiluteata</i> (MG014839)	0.095	0.086	0.110	0.078	0.084	0.106	0.120	0.122	0.086	0.106	0.091	0.084	0.008	0.004	0.006	0.006													
<i>Tanaorhinus rafflesii</i> (HM387094)	0.095	0.086	0.106	0.080	0.082	0.101	0.120	0.127	0.080	0.097	0.074	0.074	0.055	0.055	0.053	0.057	0.051												
<i>Tanaorhinus rafflesii</i> (GU662818)	0.097	0.087	0.108	0.082	0.084	0.101	0.124	0.127	0.082	0.097	0.076	0.076	0.059	0.059	0.057	0.061	0.055	0.004											
<i>Tanaorhinus rafflesii</i> (GU662820)	0.097	0.087	0.108	0.082	0.084	0.101	0.124	0.127	0.082	0.097	0.076	0.076	0.059	0.059	0.057	0.061	0.055	0.004	0.000										
<i>Tanaorhinus rafflesii</i> (GU662706)	0.097	0.087	0.108	0.082	0.084	0.101	0.124	0.127	0.082	0.097	0.076	0.076	0.059	0.059	0.057	0.061	0.055	0.004	0.000	0.000									
<i>Tanaorhinus rafflesii</i> (GU662754)	0.095	0.086	0.106	0.080	0.082	0.099	0.122	0.129	0.080	0.095	0.074	0.074	0.057	0.057	0.055	0.059	0.053	0.006	0.002	0.002	0.002								
<i>Tanaorhinus viridiluteata</i> (MW855166)	0.099	0.091	0.114	0.080	0.087	0.105	0.118	0.127	0.089	0.105	0.095	0.087	0.002	0.006	0.004	0.004	0.006	0.057	0.061	0.061	0.061	0.059							
<i>Tanaorhinus viridiluteata</i> (MW855164)	0.099	0.091	0.114	0.080	0.087	0.105	0.118	0.127	0.089	0.105	0.095	0.087	0.002	0.006	0.004	0.004	0.006	0.057	0.061	0.061	0.061	0.059	0.000						
<i>Tanaorhinus viridiluteata</i> (MW855165)	0.099	0.091	0.114	0.080	0.087	0.105	0.118	0.127	0.089	0.105	0.095	0.087	0.002	0.006	0.004	0.004	0.006	0.057	0.061	0.061	0.061	0.059	0.000	0.000					
<i>Tanaorhinus kira</i> (MG014834)	0.114	0.078	0.072	0.080	0.099	0.103	0.091	0.097	0.097	0.095	0.082	0.105	0.114	0.118	0.112	0.114	0.114	0.097	0.099	0.099	0.099	0.097	0.116	0.116	0.116				
<i>Tanaorhinus reciprocata</i> (MG014837)	0.099	0.061	0.112	0.067	0.086	0.095	0.131	0.127	0.087	0.097	0.087	0.084	0.099	0.095	0.097	0.095	0.093	0.097	0.099	0.099	0.099	0.097	0.099	0.099	0.106				
<i>Tanaorhinus luteivirgatus</i> (MG014835)	0.097	0.078	0.099	0.067	0.080	0.093	0.101	0.122	0.093	0.078	0.080	0.086	0.097	0.099	0.097	0.095	0.101	0.101	0.105	0.105	0.105	0.103	0.097	0.097	0.099	0.080			
<i>Chlorozancle falcatum</i> (MG014741)	0.112	0.097	0.097	0.082	0.089	0.108	0.118	0.120	0.097	0.105	0.099	0.101	0.099	0.105	0.101	0.103	0.101	0.110	0.114	0.114	0.114	0.112	0.099	0.099	0.099	0.105	0.087		



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