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'Fake widespread species': a new mangrove *Thinophilus* Wahlberg (Diptera, Dolichopodidae) from Bohol, Philippines, that is cryptic with a Singaporean species

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Abstract. We here show an example of how a supposed 'wide-spread' species can actually be revealed as a 'long-distance' cryptic species complex. During a recent survey of the insect fauna of the mangroves of the San Vicente Mangrove Forest Association (SAVIMA) in Bohol, Philippines, many specimens of Dolichopodidae were collected. They were pre-sorted into putative species (3% threshold) using COI sequences obtained via next-generation-sequencing (NGS barcodes: 313 bp). The sequences were then compared to a database of sequences including more than 20,000 Southeast Asian dolichopodid specimens belonging to more than 300 species. The morphology for one such Boholano putative species cluster was superficially indistinguishable from and initially identified as *Thinophilus comatus* Grootaert, 2018, described and only known from the mangroves of Singapore. However, the 4.6% divergence in sequences between the Singaporean and Boholano specimens prompted a morphological re-examination which revealed minute differences in the male terminalia. The cryptic species from the Philippines is described and illustrated here as *Thinophilus reizlae*, new species.

Key words. new species, *Thinophilus*, mangrove, Philippines, cryptic species, NGS barcodes

INTRODUCTION

The genus *Thinophilus* Wahlberg, 1844, is a globally widespread genus of Dolichopodidae (long-legged flies) that is primarily found in marine coastal environments, with most species preferring mangrove regions and having locally restricted distribution ranges for each species. Presently, 54 species are known from Southeast Asia (Grootaert, 2018); the fauna has recently been treated in a series of papers providing an overview of the species per region (Grootaert & Meuffels, 2001; Evenhuis & Grootaert, 2002; Grootaert et al., 2015; Grootaert, 2017, 2018; Samoh et al., 2017, 2019; Ramos et al., 2018), with an overview of the distribution per region and the species groups given in Grootaert (2018). Of the 54 species, only six are previously reported from the Philippines: *T. diminuatus* (Becker, 1922); *T. indigenus* Becker, 1902; *T. tesselatus* (Becker, 1922); *T. aequalichaetus* (Parent, 1941), as well as *T. lungeosetole* Ramos & Grootaert, 2018, and *T. ronazeli* Ramos & Grootaert, 2018 (in Ramos et al., 2018). Recently, we found some *Thinophilus* specimens from Bohol, Philippines that were superficially indistinguishable in morphology and thus initially identified as *Thinophilus comatus* Grootaert, 2018 — a species described and (currently) only known from Singapore. Molecular taxonomy analysis informed us that the Boholano specimens were genetically distinct, prompting a further morphological re-investigation that eventually revealed minute differences in male terminalia between these two cryptic species.

While the phenomenon of cryptic species is well-recognised (Bickford et al., 2007), we are nonetheless surprised to observe a cryptic species relationship over such a long distance apart: most *Thinophilus* species appear to be locally endemic in their own ranges within Southeast Asia (Grootaert, 2018), and Bohol Island and Singapore are almost 2,500 km apart, separated by several seas such as the South China Sea and the Sulu Sea, with distances between adjacent landmasses ranging 250–500 km on average (Fig. 1). Furthermore, extensive sampling in Brunei (which lies roughly midway between Singapore and Bohol; also Fig. 1) failed to yield any *Thinophilus* specimens similar to *T. comatus* or the new species, suggesting the possibility of a long-distance disjunct distribution within these two species (see Discussion for more details). This case calls into question the validity of apparently widespread species, where some could actually be 'long-distance' cryptic species complexes in disguise. This also highlights the importance for molecular data in helping discover such 'fake widespread species' when they are present.

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Fig. 1. Map of Southeast Asia indicating populations of *Thinophilus reizlae*, new species, in Bohol, Philippines and *T. comatus* from Singapore, as well as Brunei, where no *Thinophilus* specimens similar to either of the two species were found.

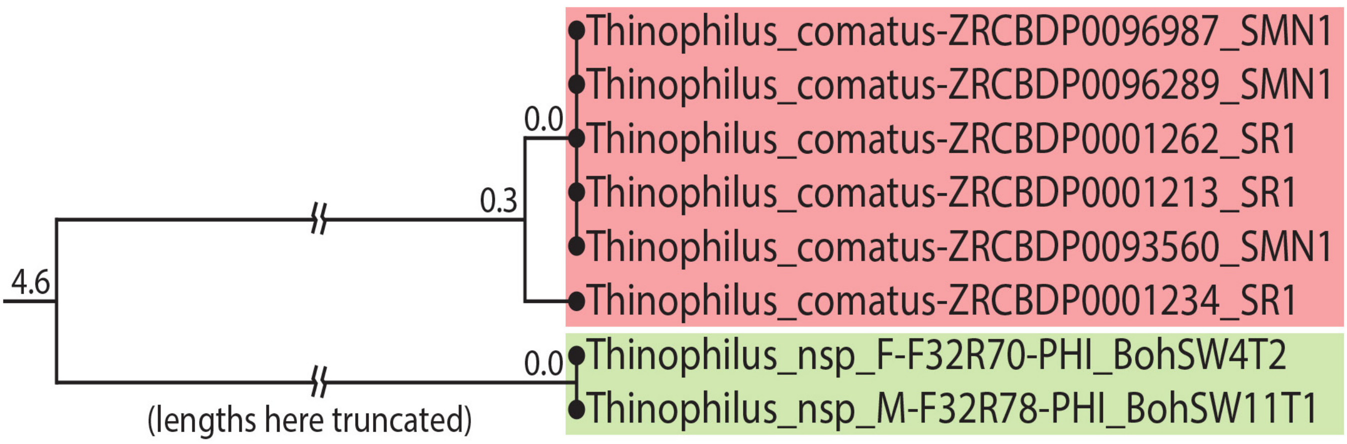

Fig. 2. Cluster dendrogram of COI (313 bp) barcodes of *Thinophilus comatus* from Singapore and *T. reizlae*, new species, from Bohol, Philippines. Objective clustering of barcodes was performed based on uncorrected p-distances using the 'best close match' criteria; the number at each 'node' represents the percentage divergence or pairwise distance threshold at which clusters split or lump together. Each cluster identified as a species is highlighted in one colour per respective species. Sequences of *T. reizlae*, new species, are highlighted in green, those of *T. comatus* in red.

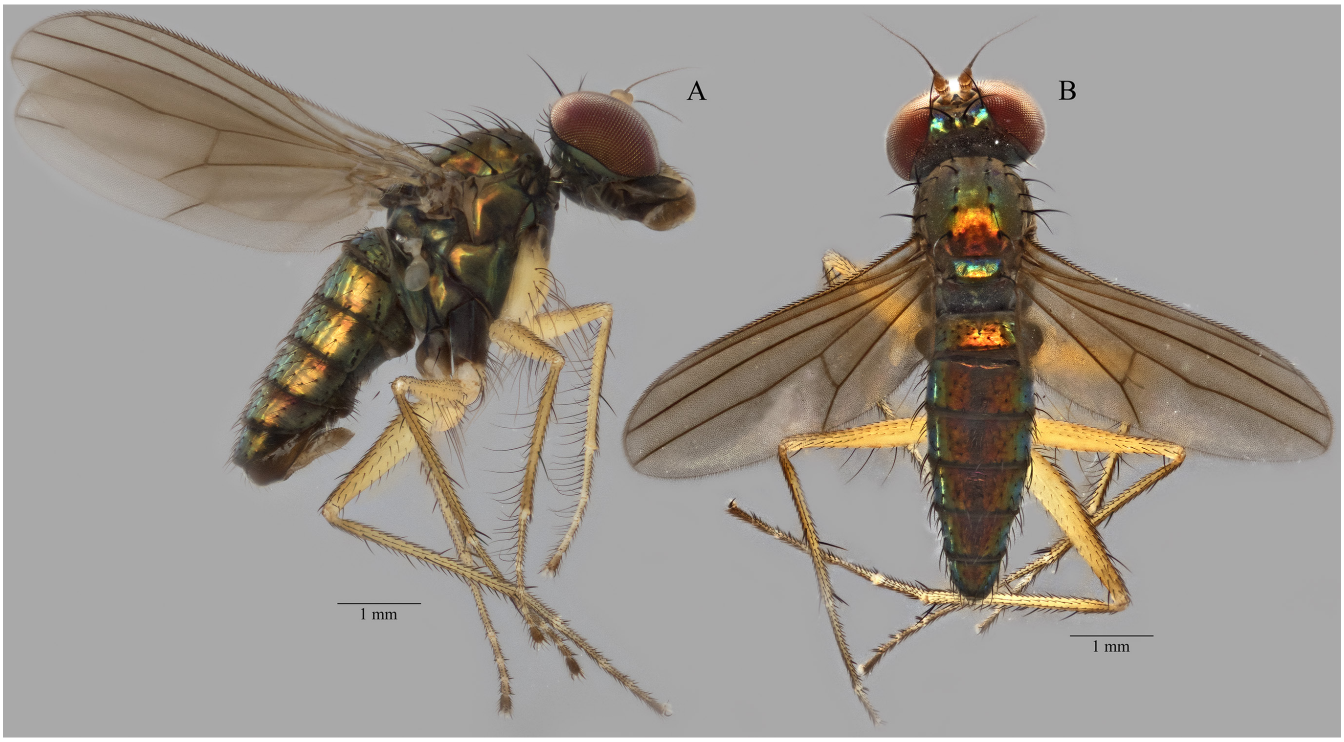

Fig. 3. *Thinophilus reizlae*, new species, holotype [specimen code UPLBMNH DIP-02425, collection code: BohSW11T1_F32_R78] male habitus. A, lateral view; B, dorsal view.

In view of the large geographic distance between the known Singaporean and Boholano populations, it is unlikely that genetic exchange can occur between them. This has probably resulted in a genetic drift which is evidently morphologically expressed, albeit weakly: there are small differences in the chaetotaxy of the male terminalia. The two populations were also resolved into two clusters stable up to 4.6% when their COI barcodes were objectively clustered (Fig. 2; see Meier et al., 2006). This is the first time that we have observed morphologically cryptic populations with a reasonably large DNA barcode divergence (4.6%) occurring in likely disjunction over such a large geographical distance. Based on these two avenues of evidence, we consider the two populations as distinct species, and describe the Boholano population as *Thinophilus reizlae*, new species.

MATERIAL AND METHODS

The material was collected with Malaise traps in the mangrove of San Vicente Mangrove Forest Association (SAVIMA) Bohol, Philippines as described in Ramos et al. (2018). The NGS-based DNA barcoding and bioinformatics as well as imaging of specimens were conducted as described in Ramos et al. (2018), based on the procedures prescribed by Meier et al. (2016). The resultant cluster fusion diagram is shown in Fig. 2. Specimens are deposited in University of the Philippines, Los Baños Museum of Natural History (UPLBMNH) and the Lee Kong Chian Natural History Museum, Singapore (ZRC). COI DNA barcode sequence for holotype specimen is uploaded onto Genbank [MN609847].

TAXONOMIC ACCOUNT

Family Dolichopodidae Latreille, 1809

Subfamily Hydrophorinae Lioy, 1864

Thinophilus Wahlberg, 1844

Thinophilus reizlae, new species
(Figs. 3, 4, 6A–C, 7A)

Material examined. Holotype: male. Philippines, Bohol, SAVIMA Mangrove. 9.730240°N, 123.853148°E, 03 September 2016; (specimen code UPLBMNH DIP-02425, collection code BohSW11T1_F32_R78; conserved in ethanol, deposited at UPLB).

Paratype: 1 female, 9.729968°N, 123.853449°E; 16 July 2016; (specimen code ZRCENT00001019, collection code BohSW4T2_ F32_R70; conserved in ethanol, deposited in ZRC).

Diagnosis. Habitus-wise, *Thinophilus reizlae*, new species, is very similar in external morphology (Figs. 3, 4) to the Singaporean species *T. comatus* (Fig. 5). The two morphological differences are mainly in the male terminalia as can be seen by comparing Fig. 6C vs. 6D and Fig. 7A vs. 7B. Firstly, in *T. reizlae* (Fig. 6C) the dorsal bristle (db) on the surstylus is much closer to the base of the apical fork of the surstylus as designated by the inner-lateral bristle (lb), being thinner than the width of the surstylus at the base of the apical fork (green measure); comparatively, in *T. comatus* (Fig. 6D) the distance is much larger, more than

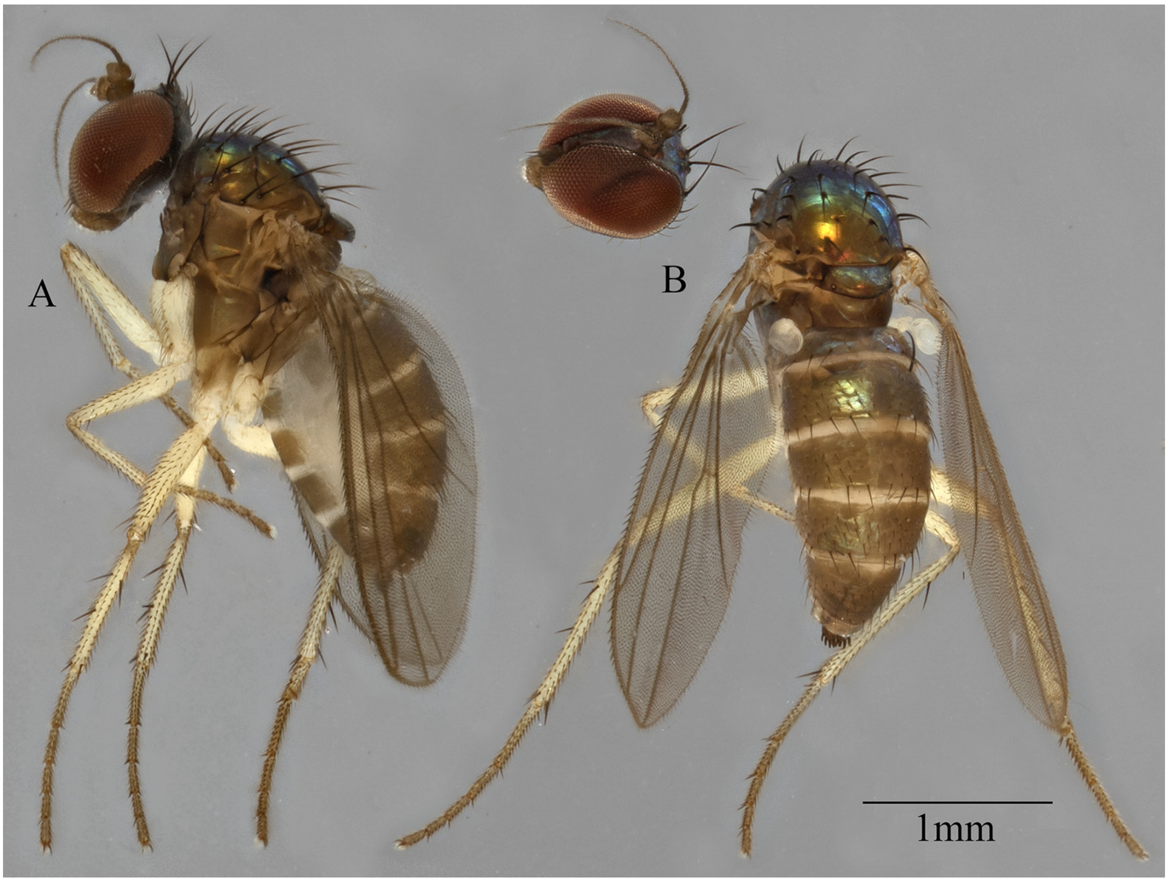

Fig. 4. *Thinophilus reizlae*, new species, paratype [specimen code ZRCENT00001019; collection code BohSW4T2_ F32_R70] female habitus. A, lateral view; B, dorsal view (separated head capsule in oblique view).

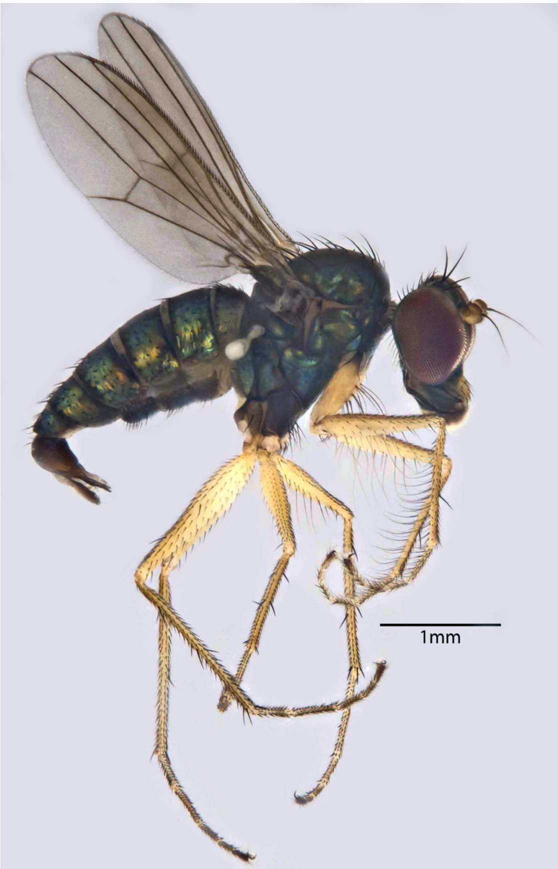

Fig. 5. *Thinophilus comatus* Grootaert, 2018, male habitus, lateral view.

the thickness of the surstylus at the base of the apical fork (red measure). Secondly, in T. *reizlae* (Fig. 7A) the ventral apical border of the hypandrium (hyb) is a distinct pointed projection (green arrow), unlike in *T. comatus* (Fig. 7B), which is a weaker, rounded swelling (red arrow).

Etymology. The species is dedicated to Reizl Jose who helped with the survey of the dolichopodid flies of Bohol Island.

Description. Male (body 3 mm; wing 2.5 mm). Antenna yellow but brownish dorsally. Postpedicel heart-shaped, slightly taller than long. Lower postoculars white, uniseriate and not developed into a favoris (= 'whisker').

Scutum with 6 dc: first dc half as long as second, 4–5 equally long, prescutellar longest and lateral to the row. Upper and lower episternal bristles pale yellowish brown.

Legs yellow, all apical tarsomeres brown. Fore coxa with long black curved bristles on anterior side, with the basal bristle longer than coxa. Fore coxa yellow but darkened at base posteriorly, mid and hind coxae black. Fore femur with very long ventral bristles with wispy apices, basal one longest and almost four times as long as femur is wide; the bristles becoming shorter towards tip, but there still 2.5–3 times as long as femur is wide. Fore tibia ventrally with a double row of long fine bristles: bristles in the anterior row about twice as long as tibia is wide, those in the posterior (posteroventral) row more than three times as long as tibia is

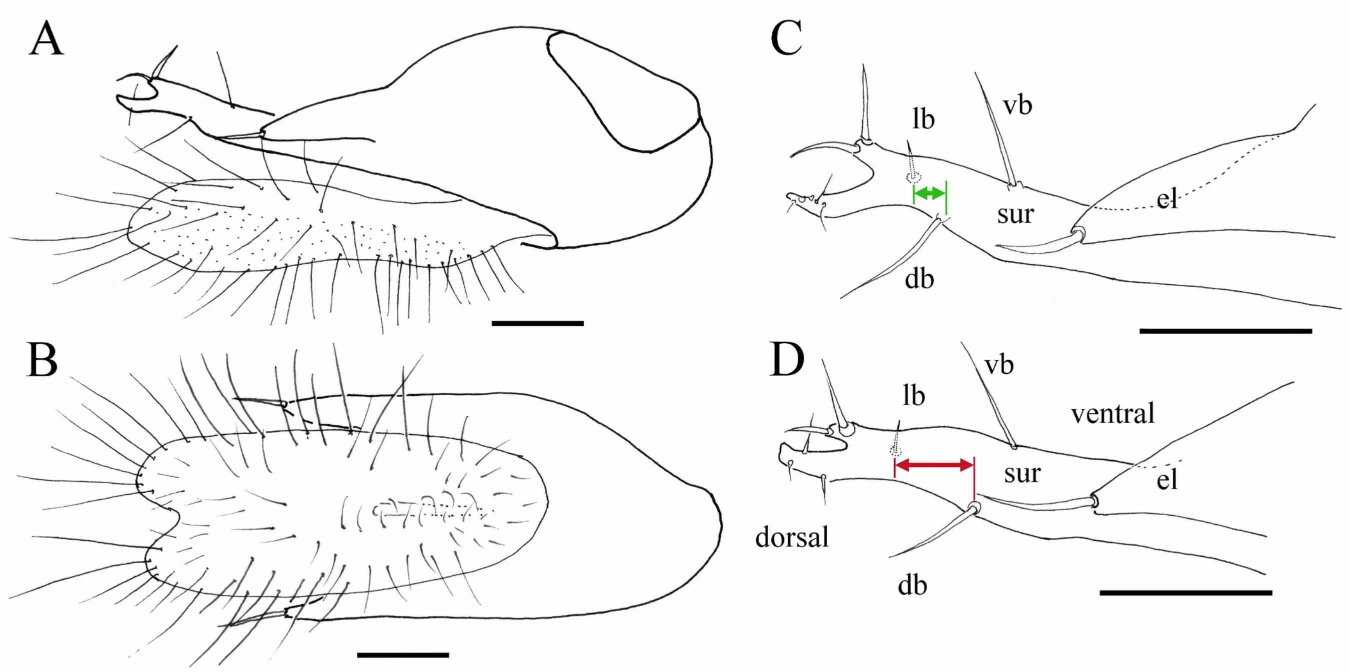

Fig. 6. A, *Thinophilus reizlae*, new species, holotype male terminalia lateral view; B, terminalia dorsal view; C, surstylus and epandrial lobe, lateral view; D, *Thinophilus comatus* Grootaert, surstylus and epandrial lobe, lateral view. Green and red measures indicate the distance between the distal (db) and inner lateral (lb) bristles for *T. reizlae* and *T. comatus* respectively. Abbreviations: db, dorsal bristle; el, epandrial lobe; lb, lateral bristle; sur, surstylus; vb, ventral bristle. Scales represent 0.1 mm.

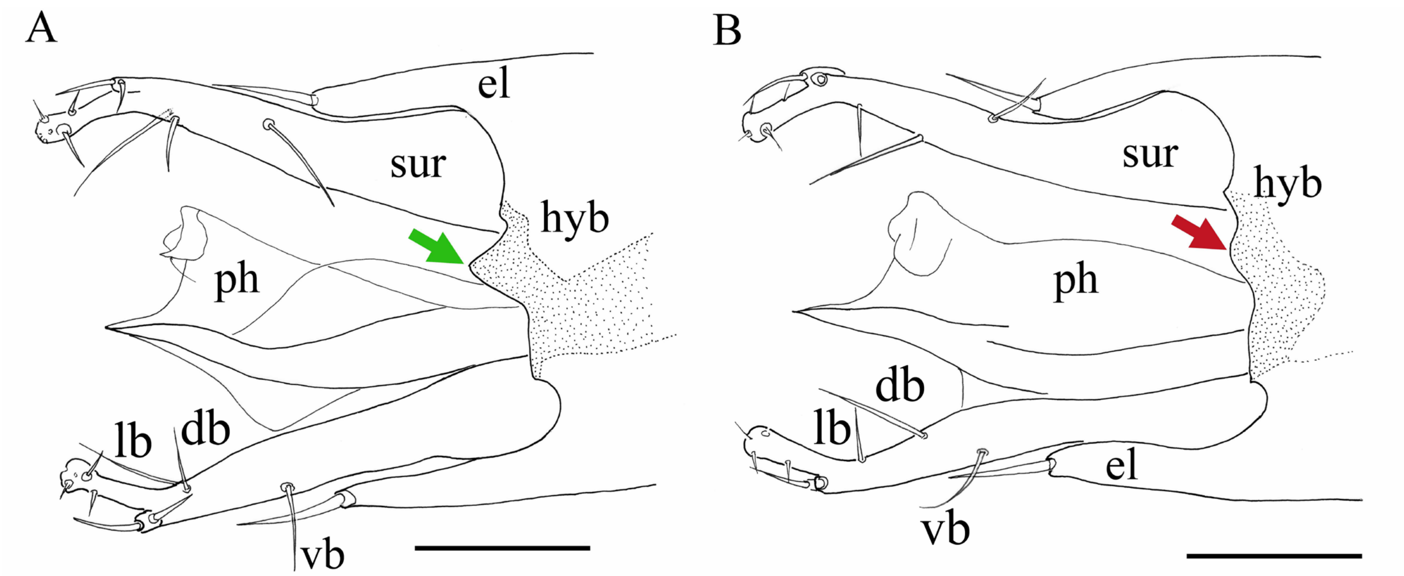

Fig. 7. A, *Thinophilus reizlae*, new species, holotype male terminalia ventral view; B, *Thinophilus comatus* Grootaert, male terminalia ventral view. Green and red arrows indicate the protrusions on the margin of the hypandrial bridge (hyb) for *T. reizlae* and *T. comatus* respectively. Abbreviations: db, dorsal bristle; el, epandrial lobe; hyb, hypandrial bridge; lb, lateral bristle; ph, phallus; sur, surstylus; vb, ventral bristle. Scale = 0.1 mm.

wide. This row is continuous onto tarsomeres 1–3. Mid femur with about five long black bristles ventromedially; shortest about 1–1.5 times as long as femur is wide; longest 2–2.5 times as long. Hind femur with only minute ventral bristles.

Wing brownish tinged. Haltere and calypter white.

Sternites with short but distinct black bristles.

Male terminalia as in Figs. 6A–C, 7A. Surstylus long dark brown, well separated from the epandrial lobe that has a thick apical bristle. Surstylus bifurcates near apex into two non-equal arms, with the distinctly shorter surstylar arm ventral and outer to the longer arm (Fig. 7A). The shorter arm bears two short bristles forming a 'V' (Fig. 6C) while the longer arm bears a pair of slender setae subapically and some minute papillae apically (Fig. 7A). At a short distance basal to the base of the surstylar fork there is a short, weak inner-lateral bristle (lb) followed shortly by a long fine dorsal bristle (db), and further down and closer to the epandrial lobe is a long fine ventral bristle (vb) (Fig. 6C). The hypandrial bridge, supposed to be the remnant of the hypandrium, bears a pointed projection medially (Fig. 7A). Cerci pale brownish, dorsally fused, delimitation of anus indistinct (Fig. 6B); set with pale long bristles on apical quarter while the bristles on the basal ¾ are brown and a little shorter.

Female (Fig. 4) similar to male except for simple chaetotaxy on the legs and the terminalia.

Barcode. Cytochrome oxidase I (COI) partial-cds (313 bp; GenBank accession code MN609847) as follows:
tatcagctggtattgcccatggaggagcatctgtagatctagctattttctcacttcatc
ttgccggagtatcatcaattctaggagctgtaaattttattacaactgtaattaatatacg
atcaaccggaattacatttgatcgtataccattatttgtttgatctgttgtaattacagcta
ttttattattactttctctaccagttttagctggagctattacaatattattaactgaccgaa
atctaaatacatcattctttgaccctgcaggaggaggggaccctattctctatcaacac
ttattt---

Remarks. Both *Thinophilus reizlae*, new species, and *T. comatus* are superficially very similar in morphology and can only be distinguished by two small male-terminalia characters. Both characters were verified in four males of *T. comatus* that were dissected and proved to have negligible variation, i.e., we consider these characters stable within *T. comatus*, and that the difference in characters for *T. reizlae* to be species-level differences and not intraspecific variation. DNA taxonomy evidence (Fig. 2) also show that *T. reizlae* is as a cluster with a significant 4.6% divergence from the Singaporean *T. comatus* population. Given that *T. reizlae* is morphologically and genetically distinct, they would constitute different species under all species concepts (see discussions under Ang & Meier, 2010, 2013; Tan et al., 2010; Rohner et al., 2014; Ang et al., 2017), and we here consider *T. reizlae* a new distinct species.

DISCUSSION

The case of *Thinophilus reizlae*, new species, and *T. comatus* adds to a growing body of evidence that apparently widespread species can actually be cryptic species complexes hidden in plain sight (e.g., Angulo & Icochea, 2010; Warner et al., 2015; Ito et al., 2019; Schäffer et al., 2019). In this case, the cryptic species complex was quickly detected due to the molecular data that was provided alongside the specimens during the morphological examination process. This highlights the importance of having a large-scale, rapid, and cost-efficient way of acquiring molecular data in biodiversity discovery projects, such as the NGS barcoding 'pipeline' as prescribed in Meier et al. (2016). We feel that this is especially important when working with highly diverse and abundant taxa such as insects, where one sample tends to yield hundreds—if not thousands—of specimens; the molecular data can help the researcher quickly sort and cluster the specimens (which would otherwise already fatigue the researcher) and provide information to help them focus their attention on where it is needed most, such as investigating the morphology for potentially cryptic species.

The relationship between geographic distance and genetic distance is not yet well understood in the Dolichopodidae. The distributional pattern for *T. reizlae*, new species, and *T. comatus* could be either long-distance disjunct (no intermediate species in-between) or clinal (intermediate species in-between). Thus far we only have had extensive sampling from one locality (Brunei) between these two populations, and should future sampling along the Northern coast of Borneo reveal specimens with intermediate genetic and/or morphological features; it would mean a clinal distribution for the species complex. However, we note that Philippines as an island archipelago is well-known for high endemism in its biodiversity (Treadaway, 1995; Brown & Diesmos, 2009), and this should extend to *Thinophilus* as well. Furthermore, *Thinophilus* species are known for having limited distributions: for example, the Singaporean *T. comatus* can only be found in three small patches of mangroves in Singapore, of which two are offshore islands (Pulau Ubin and Pulau Semakau) and one on the mainland (Sarimbun); and has not been recorded from other countries despite significant surveys in those countries (e.g., Thailand and Brunei, see Grootaert, 2018). It is likely that *T. reizlae*, new species, will have a similar limited distribution within the Philippines.

As such, we consider it highly likely that *T. reizlae* and *T. comatus* have disjunct distributions, which would then make it the first known instance of a long-distance, morphologically cryptic species pair within the Dolichopodidae with such a significant DNA barcode divergence (4.6%). As cryptic species, we managed to find only two morphological differences in male terminalia (chaetotaxy of the surstyli and structure of the hypandrial bridge) between the two species. While we note that we have only one male specimen to base our morphological data for *T. reizlae*, new species, we find that there is negligible intraspecific variation for these characters within the Singaporean *T. comatus* (n=4), which suggests that these characters would also be stable in the Boholano population. Nonetheless, additional male specimens of *T. reizlae*, new species, would be highly useful for confirming whether these characters are also stable within the Boholano population.

With the inclusion of this new species, the species count for *Thinophilus* (n=7) likely remains a gross underestimate of its species diversity within the Philippines and—especially given that they tend to have limited distributions—we are certain that many more species will be discovered once more mangroves and other coastal environments are surveyed in the Philippines.

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