A New Species of Mayfly (Ephemeroptera: Trichorythidae) from Mindanao Island, Philippines and Association of Life Stages Using DNA Barcode*

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KEY WORDS :

Mt. Malindang
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ABSTRACT

A new mayfly species, *Sparsorythus buntawensis* sp. nov. (Tricorythidae) from Layawan River of Mt. Malindang in Mindanao Island, the Philippines is described based on nymphal and adult morphologies. *Sparsorythus buntawensis* sp. nov. differ from all known members of the genus primarily in the deeper cleft of the hypopharynx and wider distance between compound eyes. Conspecific specimens of various life stages and sexes of this new mayfly were associated using DNA barcode.

INTRODUCTION

The species diversity of mayflies (Insecta: Ephemeroptera) in the Philippines, like in the whole Oriental region, is largely underestimated due to insufficient collecting effort and the region being largely "unknown lands" (Barber-James et al., 2008). Hubbard & Pescador (1978), cataloguing only 20 Philippine mayfly species, suggested that the low species number in the archipelago did not necessarily reflect a low species diversity but may be caused by sporadic and inadequate taxonomic works. From the first collecting record made by Walker (1853) in Luzon to the most recent publication of five new species by Braasch (2011) in Palawan, only a total of 37 mayfly species were described from the Philippine archipelago. They are classified into eight families: Baetidae, with 11 species from five genera; Caenidae, with four species from two genera; Heptageniidae, with 16 species from six genera; Prosopistomatidae, with two species from a single genus; and one species each for Ephemeridae, Leptophlebiidae, Teloganodidae, and Tricorythidae.

From a recent survey of aquatic insects conducted in Mindanao Island of the Philippines, a previously unknown mayfly species have been discovered, a new *Sparsorythus* (Tricorythidae). The Tricorythidae are widespread in Southeast Asia and the Indian subcontinent, and currently encompass eight described species (Barber-James et al., 2013) within the genus *Sparsorythus*. Recently revised by Sroka & Soldán (2008) based on Ulmer's (1913) *Tricorythus jacobsoni* from Java Island, *Sparsorythus* is considered as phylogenetically derived within the Tricorythidae due to its morphological apomorphies. *Sparsorythus* differs from other tricorythids by its reduced maxillary palps in nymphs, fully merged penial lobes without auxiliary processes, and a well–developed paracercus in the imagines (Sroka & Soldán, 2008). Ulmer's (1924) record of *Sparsorythus jacobsoni* in Luzon along with Sroka & Soldán's (2008) described yet unnamed *Sparsorythus* sp. 4 from Mt. Apo of Mindanao Island are the only two currently recognized tricorythids in the Philippines.

Based on the newly collected materials from Layawan River of Mt. Malindang on Mindanao Island, this study described one new mayfly species, *Sparsorythus buntawensis* sp. nov. (Tricorythidae). We associated sexes and life stages of conspecifics using DNA barcode (Gattolliat et al., 2015; Webb et al., 2012; Zhou et al., 2009; Ball et al., 2005).

MATERIALS AND METHODS

Layawan River is characterized by a rocky riverbed with large boulders, dense riparian vegetation upstream, and

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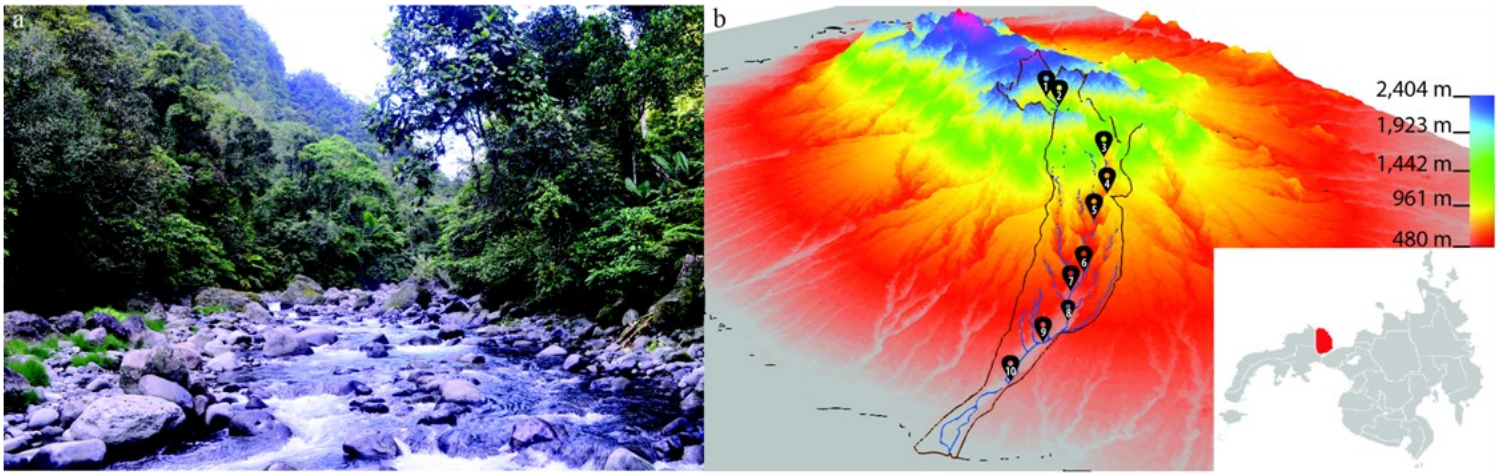

Figure 1: (a) The habitat of Layawan River, and (b) a modified 3D map of Mt. Malindang (ICRAF, 2013) showing the river system and sampling sites. Inset is Mindanao Island located in southern Philippines, the region in red is the location of Mt. Malindang Range.

farmlands downstream (Fig. 1a). This river drains into the Oroquieta watershed and is one of the major rivers in the Mt. Malindang Range Natural Park (Hansel et al., 2006), the only representative natural forest remaining in the Zamboanga Peninsula biogeographic zone of Mindanao Island (Arances, 2006) and newly designated as an ASEAN Heritage Park. The region where the river lies has a Type II Philippine climate characterized by no dry season with very pronounced rainy season from December to February (PAGASA, 2015). Ten sites (Fig. 1b) ranging from 73 to 1,215 m elevation along the stretch of Layawan River were sampled. Two sampling efforts were conducted, October 1–6, 2013 and April 13– 18, 2014. Nymphs were collected manually while adults were collected through LED light traps. All field–collected specimens were stored in 90% alcohol, then transferred to 95% alcohol in the laboratory, and finally stored at –20 ºC freezers. Nymphal specimens were carefully dissected and slide–mounted using Bioquip Euparal mounting medium. Morphological observations and imagery were made using a Nikon EOS700d digital camera mounted on an Olympus SZ61 stereomicroscope with 10 to 40x magnification. From the images captured, measurements of mayfly body parts were made using ImageJ v1.46r software (National Institutes of Health, Bethesda, MD, USA).

Genomic DNA was extracted from the legs of the insects using a standard phenol–chloroform protocol (Lin & Wood, 2002). To amplify the fragments of cytochrome c oxidase subunit I (*COI*) gene of animal DNA barcode, the forward LCO1490 and reverse HCO2198 primer (Folmer et al., 1994) were used for the Polymerase Chain Reaction (PCR). Each PCR reaction contained 1 µL of DNA, 1 µL of *Taq* polymerase, 2 µL of forward and reverse primer, 4 µL of dNTPs, 5 µL of buffer, and 35 µL of double–distilled water. The PCR procedure included: one minute of denaturation at 94 ºC, followed by 45 seconds of annealing at 53 ºC, one minute of extension at 72 ºC (35 cycles), and 10 minutes of final extension at 72 ºC. PCR products were visualized on 1.5% agarose gels and purified by shrimp alkaline phosphatase/exonuclease I, and then subjected to DNA sequencing using the BigDye® terminator 3.1 sequencing kit on an ABI 3730XL DNA Analyzer.

The forward and reverse *COI* sequences were assembled using Seqman v7.1.0 (DNASTAR, Madison, USA). All metadata and sequences >600 bps were uploaded to the Barcode of Life Database (BOLD, www.boldsystems.org) and submitted to GenBank (Table 1). Sequence analyses were carried out using MEGA 6 (Tamura et al.., 2013). Pairwise distances were calculated using Kimura–2–parameter model with pairwise deletion for gap handling and 1000 bootstrap replications to determine the genetic distance between conspecific individuals.

TAXONOMY

Order	Ephemeroptera
Family	Tricorythidae Lestage, 1942
Genus	*Sparsorythus* Sroka & Soldan, 2008
Type species	*Sparsorythus bifurcatus* Sroka & Soldan, 2008

Sparsorythus buntawensis Batucan, Nuñeza, & Lin *sp. nov.* (Figs. 2–17).

Nymph Holotype: body generally light brown to pale yellow with black dorsal and ventral markings (Fig. 2). Margins of head, abdomen, and legs yellow to pale yellow. Body length 5–6 mm. Cerci & paracercus 1.2 and 1.3 times as long as body.

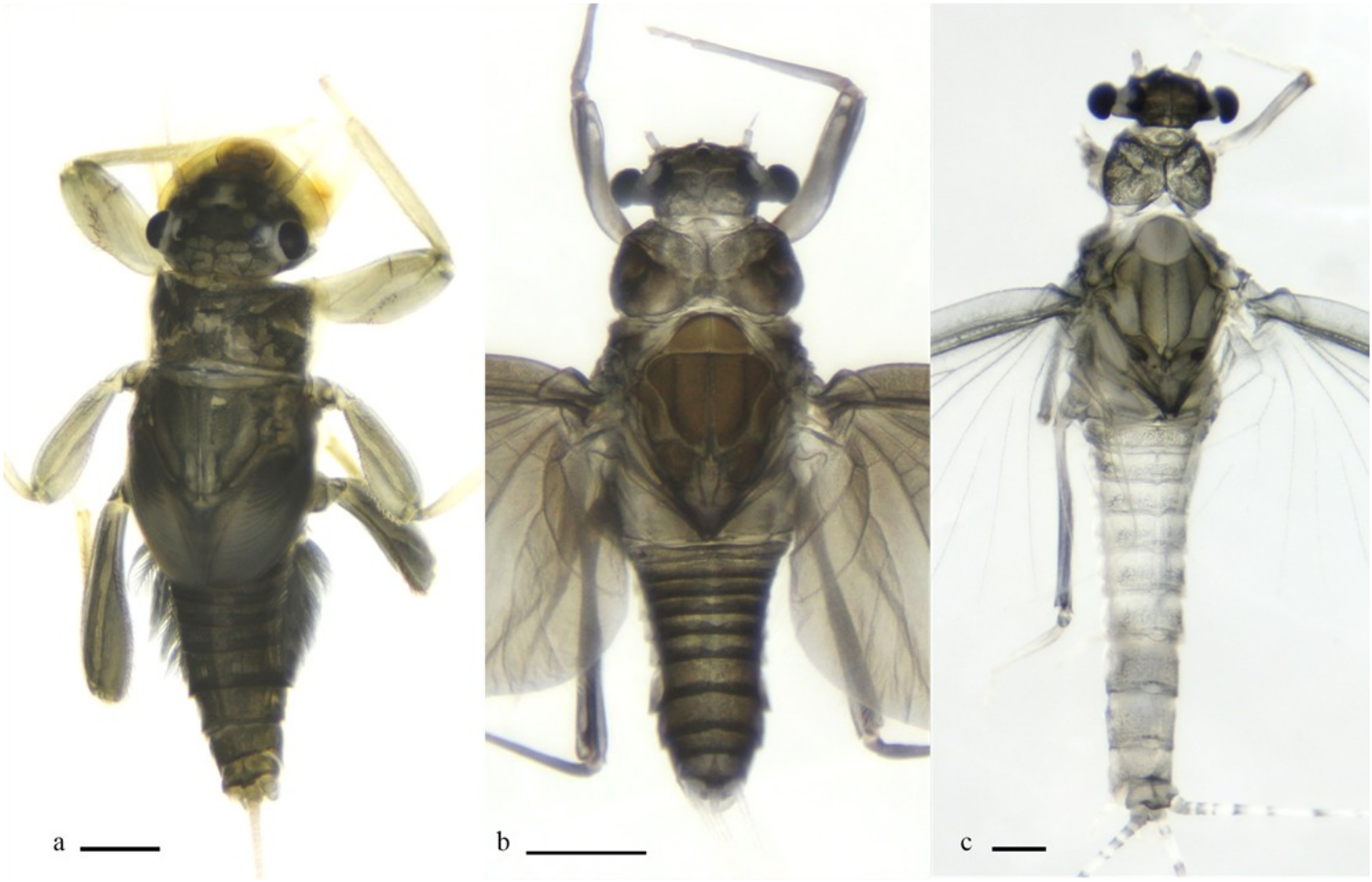

Figure 2: *Sparsorythus buntawensis* sp. nov. (a) nymph, (b) female adult, and (c) male adult. Scale ½ mm.

Head: Width to length ratio 1:1.4 (Fig. 3). Male head (Fig. 3a) with median emargination posteriorly, more ovate than that of female (Fig. 3b). Eyes black, ocelli black and smooth–edged, triangle to bean–shaped. Compound eyes of males and females approximately of same size. Ratio of distance between compound eyes and eye width 5.2:1.

Mouthparts: Labrum (Fig. 4a) twice wider than long. Hypopharynx (Fig. 4b) 1.3 times as wide as long with a deep median cleavage. Right prostheca (Fig. 4c) notched, triangular, with several short pointed teeth and ½ the length of inner incisor. Left prostheca (Fig. 4d) rod–like, pointed and notched at apex, ¾ the length of outer incisor. One stout setae near base of left prostheca, as long as right prostheca. Maxilla (Fig. 4e) without palps. Labial plate (Fig. 4f) without invagination at mid-anterior margin. Numerous basal setae on labial plate as long as lateral setae at second segment of labial palp.

Thorax and Abdomen: Gills (Fig. 5) present from segments II to VI, dorsal lamellae on segment VI (Fig. 5e) with tiny, short, marginal bristles. Legs (Fig. 6) robust. Length ratio (femur: tibia: tarsus): foreleg (Fig. 6a) 2.7:3:1, middle leg (Fig. 6b) 2.9:2.7:1, hind leg (Fig. 6c) 3.2:2.8:1. Ratio of femur length to width is 2:1, 2.1:1, and 2.2:1 for fore, middle, and hind legs, respectively. Fore femora (Fig. 6a) with black pointed setae running halfway along depression on upper surface, directed apically and interspersed with short yellow setae, upper margin with setae medially subequal in length as upper surface setae, setal length and point variable. Ventral margin of fore tibia (Fig. 6a) with fine long hairs and one stout apical pointy setae, dorsal margin with shorter and thinner hairs. Dorsal surface of middle and hind femora (Fig. 6c) with very small stout setae, absent in depression. Mid- and hind femora longer than tibia. Ventral margin of middle tibia (Fig. 6b) with small and blunt setae, apex with two pointed setae. Dorsal margin of middle and hind femora (Fig. 6b, 6c) with stout and long blunt setae, irregularly alternating in length, running medial distally. Dorsal margin of hind tibia (Fig. 6c) have similar setae but more pointy ends. Caudal filament segments (Fig. 7) surrounded with short, thin setae having maximum length approximately 1/3 of segment.

Male Imago Paratype: Body length 4–5 mm. General coloration light black to gray. Cerci twice longer than body, paracercus approximately 2.6 times longer than body length.

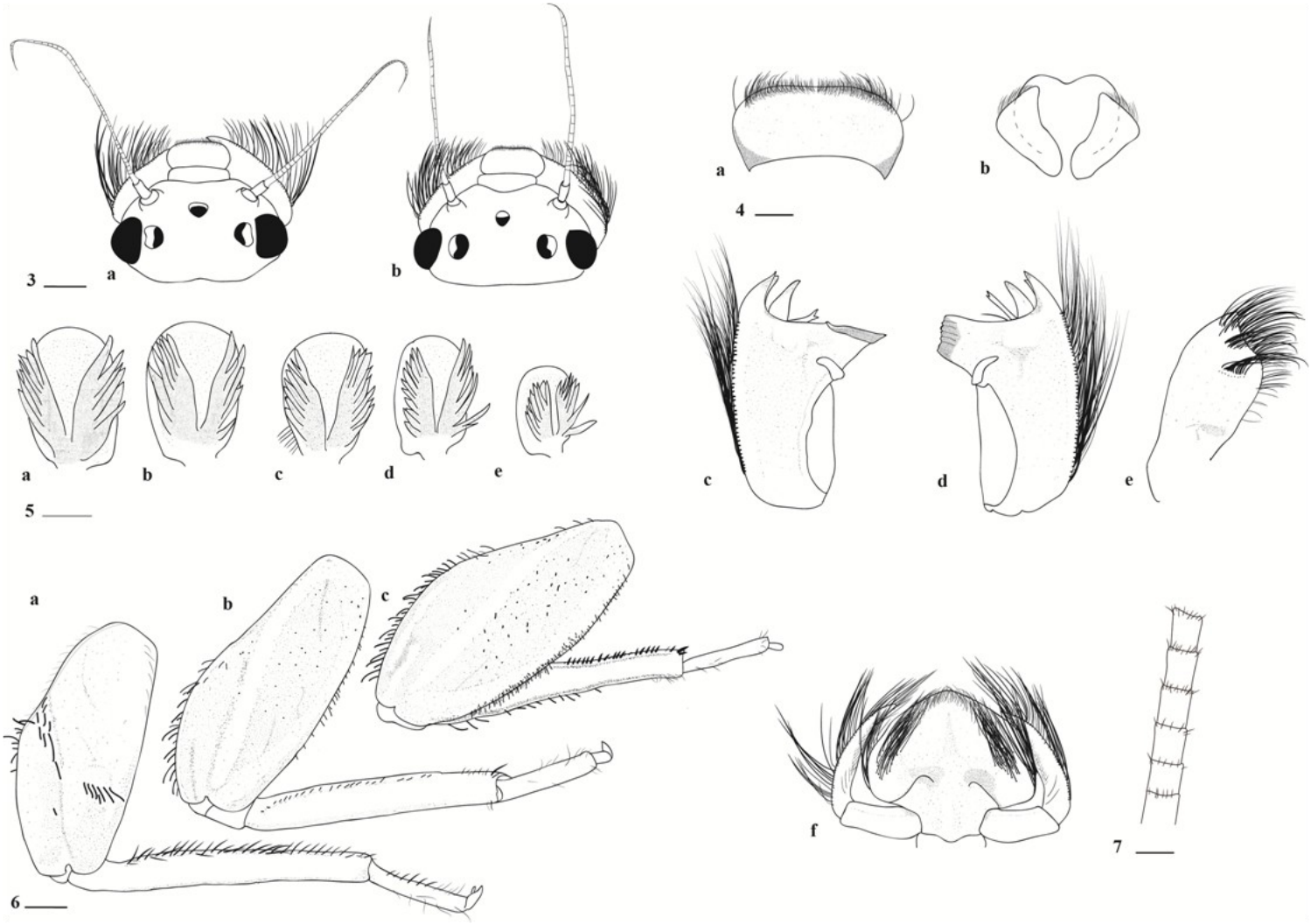

Figure 3: Nymph of *S. buntawensis* sp. nov. dorsal view of head (scale ¼ mm), (a) male, (b) female.
Figure 4: Dorsal view of mouthparts of nymphs (scale 1/8 mm), (a) labrum, (b) hypopharynx, (c) right mandible, (d) left mandible, (e) maxilla, (f) labium.
Figure 5: Ventral view of gills (scale ¼ mm), (a) II, (b) III, (c) IV, (d) V, (e) VI.
Figure 6: Dorsal view of legs (scale ¼ mm), (a) fore leg, (b) middle leg, (c) hind leg.
Figure 7: Setation of caudal filament (scale 1/10 mm).

Head: Gray to black, width 1 mm (Fig. 8). Compound eyes approximately of same size as those of females. Ratio of distance between compound eyes and eye width, 5:1. Scape to pedicel ratio 1:2.

Thorax and Abdomen: Prothorax gray to black with brown to white blotches. Mesothorax and metathorax brown, gray to black, darker coloration at junctions of segments. Femur gray to black, tibia and tarsus gray to brown. Cerci white, segment junctions gray to black. Segments of paracercus twice the length of cerci segments (Fig. 9). Segment I of forceps (Fig. 10) approximately 1/3 of segment II, penis lobe constricted subapically, slightly extending beyond segment I of forceps. Forewing venation gray to black, median to posterior surface transparent (Fig. 11). Leg ratio (femur: tibia: tarsus): foreleg (Fig. 12a) 2.3:2.12:1, middle leg (Fig. 12b) 2.6:2.2:1, hind leg (Fig. 12c) 3.6:3.3:1. Claws ephemeroid.

Female Adult Paratype: Body length 4–5 mm. Cerci and paracercus 1.3 and 1.5 times as long as body length.

Head: Gray to black, width 1.2 mm (Fig. 13). Ratio of distance between compound eyes and eye width, 3.4:1 (males 5:1), compound eyes slightly larger than those of males. Scape to pedicel ratio 1:2.

Thorax and Abdomen: Prothorax gray to black with brown to white blotches. Meso- and metathorax brown to black. Femur gray to black, tibia and tarsus white to brown. Caudal filaments covered with short, fine setae (Fig. 14). Subanal plate slightly pointed (Fig. 15). Venation of forewing (Fig. 16) similar to that of male, wing surface translucent. Forewing 6.5 mm long, surface of wing dark-colored anteriorly and lighter posteriorly, with microtrichia. Legs (Fig. 17) equipped with one hooked claw and one pointy claw,

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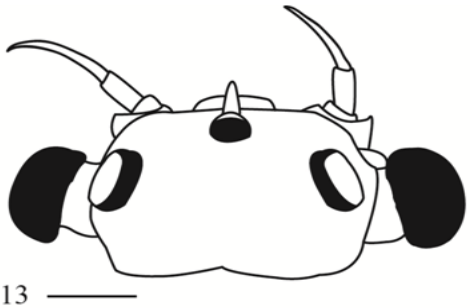

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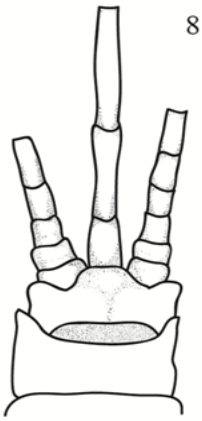

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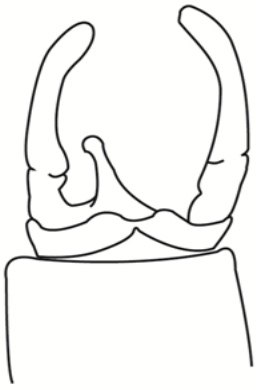

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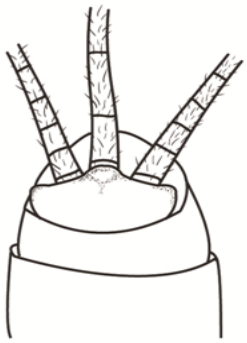

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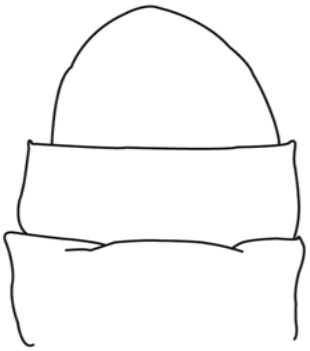

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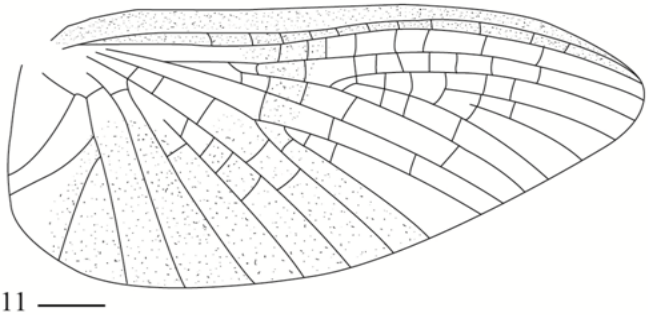

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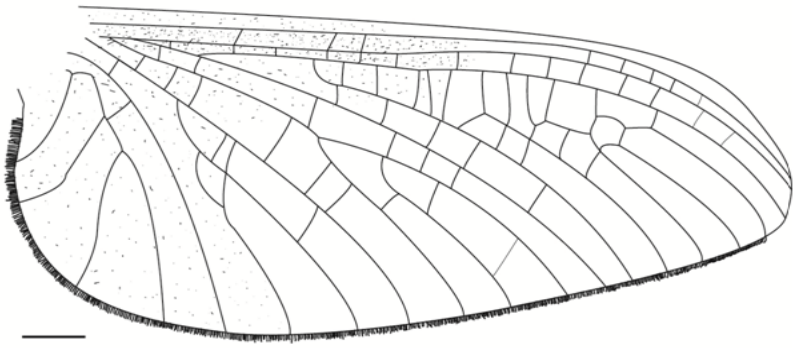

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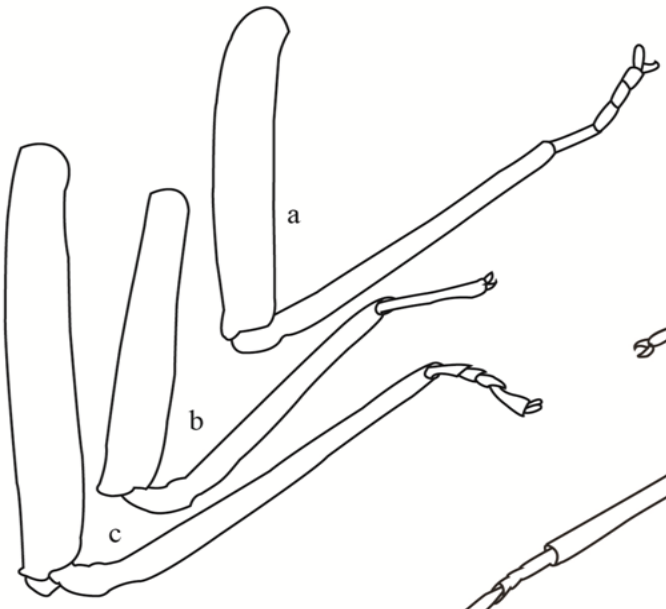

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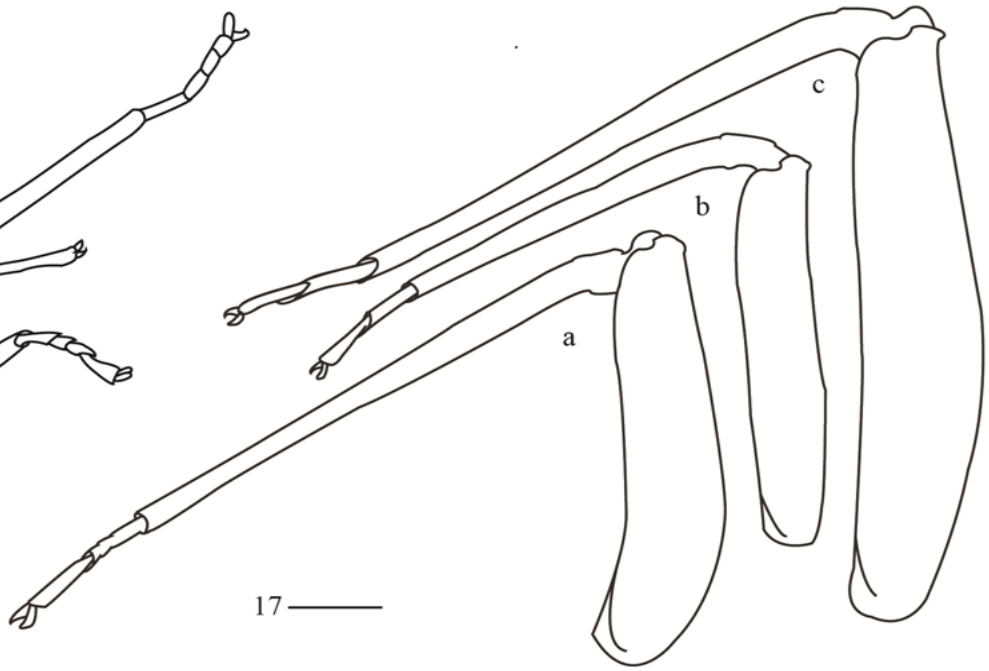

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pointy claw, femora wider and longer than those of males, leg ratio (femur: tibia: tarsus): foreleg (Fig. 17a) 3.3:4:1, middle leg (Fig. 17b) 3.7:3.3:1, hind leg (Fig. 17c) 5.7:5.2:1.

Life Stage Association: Two *COI* barcode sequences of 641 bps for each life stage were obtained. Mean intraspecific K2P sequence divergence between nymphs and adults of *S. buntawensis* sp. nov. was 10.2 ± 1.0% (n=4). Within the life stages, sequence divergence was 3.6 ± 0.7% (n=2) for nymphs and 0.6 ± 0.3% (n=2) for adults. No *COI* barcode sequences of other tricorythid species are currently available in Genbank and BOLD for comparison. Nevertheless, the average intraspecific sequence divergence of *COI* in *S. buntawensis* sp. nov. (10.2%) is much lower than the interspecific divergence (18%) for all mayfly species (13.7–25.8% for Tricorythidae's sister families, Leptohyphidae and Ephemerellidae; Ball et al., 2005), this suggests that these specimens likely belong to the same species. Also, specimens were collected at the same site and season, and no other tricorythid species were found in the area.

Etymology: The species is named after Buntawan, the locality where the species was first found.

Type Designation and Repository: Holotype: 1 nymph (parts on slides), PNM 13547, Layawan River, Upper Toliyok, Oroquieta City, Philippines, elevation 185 m, 3 October 2013, 08° 25'25.0788" N, 123°41'48.3612" E. Paratypes: 6 imagoes (in 95% alcohol), PNM 13548, PNM 13549, PNM 13550, PNM 13551, PNM13552), Layawan River, Buntawan, Oroquieta City, Philippines, elevation 73 m, 6 October 2013, 08° 27'17.3988" N, 123°44'41.8200" E; PNM 13553, Layawan River, Lower Tuminawan, Oroquieta City, Philippines, elevation 610 m, 17 April 2014 08°23'05.5572" N, 123°39'01.4220" E; 5 nymphs (in 95% alcohol), PNM 13554, PNM 13555, NM 13556, PNM 13557, Layawan River, Lower Toliyok, Oroquieta City, Philippines, elevation 161 m, 08°25'58.2600" N, 123° 42'29.5200" E; PNM 13558, Layawan River, Upper Toliyok, Oroquieta City, Philippines, elevation 185 m, 08°25'25.0788" N, 123°41'48.3612" E; deposited along with the holotype in the National Museum of Natural History, Philippine National Museum, Manila, Philippines. All types were collected and prepared by Leocris S. Batucan Jr..

←**Figure 8:** Dorsal view of *S. buntawensis* sp. nov. Head of male adult (scale: 1/16 mm).
←**Figure 9:** Male caudal filaments (dorsal, scale: ¼ mm).
←**Figure 10:** Penis (ventral, scale: 1/16 mm).
←**Figure 11:** Forewing of male (scale ½ mm).
←**Figure 12:** Legs of male imago (scale: ¼ mm
←**Figure 13:** *S. buntawensis* sp. nov. head of female adult (scale: 1/16 mm).
←**Figure 14:** Female caudal filaments (dorsal, scale: ¼ mm).
←**Figure 15:** Subgenital plate (ventral, scale: ¼ mm).
←**Figure 16:** Female forewing (scale ½ mm).
←**Figure 17:** Legs of adult females (scale: ¼ mm).

Diagnosis and Discussion: The nymph of *S. buntawensis* sp. nov. differ from known Oriental tricorythids mainly through the deeper and smoother cleft of its hypopharynx, the longer than wide superlinguae, the highest ratio of distance between compound eyes and eye width, and the largest leg length ratio. *S. buntawensis* is closest to *S. grandis* in terms of leg length ratio and the absence of medial rill on the hypophrarynx. However *S. grandis* has a more oblongate hypopharynx, shorter and wider superlinguae, and narrower and more pointy cleft whereas *S. buntawensis* is not oblongate, has deeper, wider, and blunt cleft, and a superlinguae with pronounced concavity in its inner anterior edge. The shared morphological characters between *S. buntawensis* sp. nov. and *S. ceylonicus* are the similar length of setae of caudal filaments, middle & hind femora, and eye to eye width ratio. Although Ulmer (1924) recorded imagoes of *S. jacobsoni* from the Philippines, there is no other record of *S. jacobsoni* being found in other Philippine islands and no type specimens were available for access by the authors. Based on Ulmer's (1913, 1924) and Sroka & Soldan's (2008) description, *S. jacobsoni* is generally larger than *S. buntawensis* sp. nov. It also has a small nick in the median anterior margin of its labial plate whereas this is absent in *S. buntawensis* sp. nov. In comparison with other *Sparsorythus*, *S. buntawensis* sp. nov. has a more constricted penial lobe.

The adults of *S. buntawensis* sp. nov., exhibit a marked sexual dimorphism in head size, in which males have smaller heads than do females. For the female of *S. buntawensis* sp. nov., its paracercus is shorter than the cerci. After the final molt to adult, in contrast to *S. celebensis, S. buntawensis* sp. nov. females have prominent microtrichia on the fore wings and its forelegs are equipped with two claws wherein one is hooked and the other one is pointy. Coupled with the absence of usual imaginal characteristics of the females and absence of imaginal females despite the intensive sampling, this observation leads us to conclude that the females of *S. buntawensis* sp. nov. have only one adult stage as compared to two stages in males. This non-molting female subimago is similar to the confirmed non-molting Tricorythidae such as *Tricorythus varicauda*, *T. discolor*, and *T. tinctus* (Kluge, 2010).

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Table 1. List of specimens, collecting, and sequencing information used in this study.

Specimen No.	Taxa	Life stage	Locality	Process ID	GenBank Accession
1.9.3	*Sparsorythus buntawensis* **sp. nov.**	nymph	Bunga, Mt. Malindang, Philippines / 08°26'18.8412" N, 123°43'10.4412" E	LYWN012-15	KT250141
1.8.6	*Sparsorythus buntawensis* **sp. nov.**	nymph	Lower Toliyok, Mt. Malindang, Philippines / 08°25'58.2600"N, 123°42'29.5200"E	LYWN011-15	KT250142
6M7.2	*Sparsorythus buntawensis* **sp. nov.**	male	Upper Toliyok, Mt. Malindang, Philippines / 08°25'25.0788"N, 123°41'48.3612"E	LYWN023-15	KT250143
6F10.44	*Sparsorythus buntawensis* **sp. nov.**	female	Buntawan, Mt. Malindang, Philippines / 08°27'17.3988"N, 123°44'41.8200"E	LYWN009-15	KT250144

Environmental and Natural Resources Office (CENRO) of Oroquieta for providing guides during sampling, and the Department of Environment and Natural Resources' Protected Area Management Board Region X for issuing a gratuitous permit (GP No. R102010–04). Gratitude is extended to the members of the Systematics and Evolutionary Biology Laboratory of NTNU, and to Pavel Sroka for giving valuable comments regarding the Tricorythidae. We also thank the two anonymous reviewers who made efforts in improving this manuscript and Robert Coombs of ICRAF for providing us a 3D map Mt. Malindang. This research was supported by the Ministry of Science and Technology of Taiwan (NSC 100–2311–B–029–004–MY3 & MOST 103–2311–B–029–001–MY3 to CPL). The authors acknowledge the National Core Facility Program for Biotechnology, Taiwan (MOST 103–2319–B–010–001) for DNA sequencing.

LITERATURE CITED

Arances, J.B., V.B. Amoroso, O.M. Nuneza, & J.A Kesslers. 2006. Participatory biodiversity assessment in Malindang Range, Philippines. *In*: Society, environment, and development: The Mt. Malindang Experience. SEAMEO SEARCA BRP, Philippines. 25 p.

Ball, S.L., P.D.N. Hebert, S.K. Burian, & J.M. Webb. 2005. Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of North American Benthological Society*, 24, 3, 508–524.

Barber–James, H.M., J-L. Gattoliat, M. Sartori, & M.D. Hubbard. 2008. Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. *Hydrobiologia*, 595, 339–350.

Barber–James, H., M. Sartori, J-L. Gattolliat, & J.M. Webb. 2013. Freshwater Animal Diversity Assessment, World checklist of freshwater Ephemeroptera species. Available from http://fada.biodiversity.be/group/show/35/ (February 24, 2015).

Braasch, D. 2011. New species of the family Heptageniidae (Ephemeroptera) from Borneo and the Philippines. *Deutsche Entomologische Zeitschrift*, 58, 2, 201–219.

Folmer O., M. Black, W. Hoeh, R. Lutz, & R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Gattolliat, J-L., E. Cavallo, L. Vuataz, M. Sartori. 2015. DNA barcoding of Corsican mayflies (Ephemeroptera) with

implications on biogeography, systematics, and biodiversity. Arthropod Systematics & Phylogeny, 73, 1, 3 -18.

Hansel, C.G., A. Gorospo-Villarino, A.B. Mohagan, S.S. Nacua, T.O. Poblete, E.C. Roa, R.A. Seronay, F.F.M. Freire, B.A. Roscom, M.M. Gorospe, K.P. Culminas, W.H.C. Gay, D.G.G Bacaltos. 2006. Assessing the Headwaters of Layawan River: Linkage Between the Terrestrial and Aquatic Ecosystems in Mt. Malindang, Misamis Occidental. *Biodiversity Research Programme for Development in Mindanao: Focus on Mt. Malindang and Environs. SEAMEO SEARCA*.

Hubbard, M.D., & M.L. Pescador. 1978. A catalog of the Ephemeroptera of the Philippines. *Pacific Insects*, 19, 91–99.

Kluge, N.J. 2010. Redescription of the taxon Tricorygnatha (Ephemeroptera, *Tricorythus* s.l.) based on new findings in Africa and Indonesia. *Russian Entomological Journal*, 19, 2, 79–104.

Lestage J.A. 1942. Contribution à l'étude des Ephéméroptères. XXV. Notes critiques sur les anciens Caenidiens d'Afrique et sur l'indépendance de l'évolution tricorythido-caenidienne. Bulletin du Musée Royal d'Historie de Belgique 18(48):1-20.

Lin, C–P., & T. K. Wood. 2002. Molecular phylogeny of the North American *Enchenopa binotata* (Homoptera: Membracidae) species complex. *Annals of the Entomological Society of America*, 95, 162–171.

Philippine Atmospheric, Geophysical, and Astronomical Services Administration. 2015. Climate of the Philippines. Available from: http://kidlat.pagasa.dost.gov.ph/index.php/climate-of-the-philippines (June 6, 2015).

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2014.

Sroka, P. & T. Soldán. 2008. The Tricorythidae of the Oriental Region. In Hauer F.R.; Stanford J.A.; Newell R.L. (eds.), International advances in the ecology, zoogeography and systematics of mayflies and stoneflies. *University of California Publications in Entomology*, 128, 313–354.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, & S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.

Ulmer, G. 1913. Ephemeriden aus Java, Gesammelt von Edw. Jacobson, Note V., *Notes from the Leyden Museum*, 35, 102–120.

Ulmer, G. 1924. Ephemeropteren von den Sunda-Inseln und den Philippinen. *Treubia* 6 1:28-91

Walker, F. 1853. List of the specimens of neuropterous insects *In:* The collection of the British Museum, *Termitidae–Ephemeridae*, 3, 477–585.

Webb, J.M., L.M. Jacobus, D.H. Funk, X. Zhou, B. Kondratieff, C.J. Geraci, R.E. Dewalt, D.J. Baird, B. Richard, I. Phillips, & P.D.N Hebert. 2012. A DNA Barcode Library for North American Ephemeroptera: Progress and Prospects. PLoS ONE. 7, 5, e38063

Zhou, X., S.J. Adamoqwicz, L.M. Jacobus, R.E. DeWalt, & P.D.N. Hebert. 2009. Towards a comprehensive barcode library for arctic life – Ephemeroptera, Plecoptera, and Trichoptera of Churchill, Manitoba, Canada. *Frontiers in Zoology*, 6, 30.