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DNA-barcoding unveils a high diversity of caddisflies (Trichoptera) in the Mount Halimun Salak National Park (West Java; Indonesia)

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Background. Trichoptera are one of the most diverse groups of freshwater insects worldwide and one of the main bioindicators for freshwater quality. However, in many areas, caddiesflies remain understudied due to lack of taxonomic expertise. Meanwhile, globally increasing anthropogenic stress on freshwater streams also threatens Trichoptera diversity.

Methods. To assess the Trichoptera diversity of the area within and around the Mount Halimun Salak National Park (MHSNP or Taman Nasional Gunung Halimun Salak) in West Java (Indonesia), we carried out a molecular-morphological study of adult and larval trichopteran diversity based on a benthic survey and hand netting. In addition to morphological identification, we applied three different species delimitation approaches (Generalized Mixed Yule Coalescent, Bayesian Poisson Tree Processes, and Automatic Barcode Gap Discovery) based on DNA-barcoding of Cytochrome-C-Oxidase I.

Results. The molecular delimitation detected 73 to 79 Operational Taxonomic Units (OTU). Only five OTUs could be identified to species level by comparing sequences against the BOLD database using BLAST, and four more to the genus level. Adults and larvae could be successfully associated in 17 cases across six families. The high diversity of Trichoptera in this area highlights their potential as bioindicators for water quality assessment.

Conclusions. This study provides an example of how molecular approaches can benefit the exploration of hidden diversity in unexplored areas and can be a valuable tool to link life stages. However, our study also highlights the need to improve DNA barcode reference libraries of Trichoptera for the Oriental region.

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Abstract

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Introduction

Trichoptera (caddisflies), with currently 16,267 described species, represent one of the largest orders of primarily aquatic insect species worldwide (Morse, 2020). In tropical regions, the high Trichoptera diversity has been linked in the past with the high variety of larval adaptations to different habitat types (Mackay & Wiggins, 1979), and high rates of endemism in mountainous areas (de Moor & Ivanov, 2008). Caddisfly larvae habitat requirements vary significantly ~~between~~ species, and they are frequently used as a bioindicator for monitoring water quality (Ab Hamid & Md Rawi, 2017; Bonada et al., 2006). This type of monitoring provides better predictions than physicochemical approaches and is less expensive (Iliopoulou-Georgudaki et al., 2003). However, it requires the availability of a good knowledge of species taxonomy in the area of interest, which is rarely the case especially in tropical and remote areas (Geraci et al., 2011; Hoppeler et al., 2016). Moreover, caddisfly taxonomy is largely based on traits of adult males, whereas larval morphology remain unknown in many species (Zhou et al., 2007). A recent estimate predicts that around 13,000 Trichoptera species are awaiting recognition as formal species (Zhou et al., 2016), which further complicates their use as bioindicators.

In Indonesia, knowledge on the caddisfly fauna remains very limited. Previous studies have revealed that Java with 146 species, is one of the most diverse islands, exceeding considerably Bali (with 73 species) and Lombok (with 61 species) (Malicky et al., 2014). The MHSNP in southern West Java has one of the last remaining sub-montane forests in this part of the island (Kahono, 2003; Whitten et al., 1996). Located near the capital city of Jakarta, it serves as the major water reservoir for this megacity (Peggie & Harmonis, 2014), since its rivers and streams have water also during the dry season. Due to its richness of habitats, ranging from lowland and lower montane rain forest to montane forest, this park belongs to the area with the highest biodiversity in Java (Kahono, 2003). Still, due to some settlements, agricultural practices, and illegal gold mining in the national park, the anthropogenic pressure on rivers increases especially during the dry season (Galudra et al., 2005; Yoga et al., 2014b). Studies of freshwater organisms inside the National Park have only been conducted sporadically (e.g. on crustaceans by Ng & Wowor, 2018, 2019, dragonflies by Aswari, 2004, or on snails by Heryanto, 2001). The only study available on aquatic insect diversity in this area recognized a total of 269 caddisfly species, including 12 families of Trichoptera (Rizali et al., 2002). However, the sampling areas, though located within the park, were highly disturbed.

DNA-barcoding takes advantage of intraspecific variability in a 658-base-pair (bp) long part of the mitochondrial gene Cytochrome-C-Oxidase I (COI), and is a valuable standard tool for studying unknown diversity, especially in groups where taxonomic expertise is ~~very rare~~ (Borisenko et al., 2009; Hebert et al., 2003). It has been used successfully to assess species diversity in understudied areas (Araujo et al., 2018; Cordero et al., 2017; Geraci et al., 2011, 2011; Janzen & Hallwachs, 2011) and provide insights into cryptic species diversity (Hebert et

al., 2004; Pauls et al., 2010; Tyagi et al., 2019). The method has in many cases facilitated the association between life stages without the time-consuming rearing of specimens (Ahrens et al., 2007; Gattolliat & Monaghan, 2010; Hjalmarsson et al., 2018; Molina et al., 2017; Ruiter et al., 2013; Zhou et al., 2007), which often lack significant visible intraspecific features (Johanson, 2007; Ruiter et al., 2013; Zhou et al., 2007), and has also turned out to be a valuable tool in stream monitoring routines (Behrens-Chapuis et al., 2021).

In this study, we assess the Trichoptera diversity of the MHSNP, apply three different species delimitation methods (bPTP, AGBD, and GMYC) to estimate the number of species entities, and associate larva and adults by DNA-barcoding as part of the Indonesian-German IndoBioSys (Indonesian Biodiversity and Information System) project. OTUs are compared with the BOLD database (Ratnasingham & Hebert, 2007) to assign species names ~~where~~ possible and to determine the number of putative species missing in genetic reference libraries. Our results contribute to a better understanding of the Trichoptera diversity in West Java.

Materials & Methods

Taxon Sampling

~~Juvenile~~ and adult trichopteran specimens were collected at 26 sampling sites between 252 and 1400 m above sea level in 2015 (dry season, September) and adults additionally in 2016 (wet season, April) (Research permit no. 339/SIP/FRP/E5/Dit.KI/IX/2015 by the Ministry of Research and Higher Education of the Republic of Indonesia). Sites inside the Mount Halimun Salak National Park in West Java, Indonesia, were selected as part of a larger biodiversity assessment study of this area (Araujo et al., 2018). Additionally, samples from the vicinity of Bogor were included. The sampling of the larval Trichoptera followed a multi-habitat sampling approach of 20 pooled sampling units along a ~~100m~~ stream stretch with the standard kick-sampling method (Barbour et al., 1999) and stone washing from down- to upstream using a dip net (standard Heberle net 25 x 25 cm frame; 2 mm mesh-sized). Adults were collected in the field by sweeping with a net or using a light trap. Specimens were preserved in 96% ethanol. Larval morphospecies were primarily identified based on the taxonomical keys of de Moor & Ivanov, 2008, Malicky et al., 2014, and Yule & Yong, 2004. The identification of adult specimens followed Malicky et al., 2011, Malicky et al., 2010, Ulmer, 1913, 1930 and 1951.

DNA extraction, amplification, and sequencing

DNA was extracted from 180 specimens using the standardized Glass Fiber Plate DNA Extraction protocol of the Canadian Center for DNA Barcoding (Ivanova et al., 2006). PCR followed the CCDB protocol and a 658-bp fragment of the mitochondrial gene COI was amplified using the primer pairs HCO2198-JJ (Astrin & Stüben, 2008). Samples were sequenced at the Canadian Center for DNA Barcoding (CCDB).

Sequence and phylogenetic analysis

Raw data was assembled and trimmed with MEGA (v.7.0; (Kumar et al., 2016) and Sequencher (v.5.0, Gene Codes Corporation, Ann Arbor, Mich). The COI haplotype sequences were combined with two selected lepidopteran outgroup species (Triodia sylvina, Accession No.: JN307373 and Dyseriocrania subpurpurella, Accession No.: HQ563464). The sequences were aligned using MUSCLE (Edgar, 2004) and edited in Geneious (v.7.1.9, Biomatters, Auckland, New Zealand). COI gene trees were reconstructed using both Bayesian inference (BI) and Maximum Likelihood (ML). The Bayesian tree was inferred using BEAST (v.1.8.3; Drummond & Rambaut, 2007)), with two independent runs from a random starting tree, an uncorrelated lognormal relaxed clock, GTR+G+I substitution model, and the Yule tree prior. The Monte Carlo Markov chains (MCMC) ran twice for 30 million generations, with sampling at every 3000 generations. Convergence was checked using Tracer v. 1.7 (Rambaut et al., 2018). The ML tree was calculated in MEGA (v.7.0; (Kumar et al., 2016) based on the GTR+G+I model (selected also in MEGA) and support was calculated using non-parametric bootstrap with 1000 replicates.

Species delimitation analysis

Three different tree-based species delimitation approaches were applied: the generalized mixed Yule coalescent model (GMYC; Fujisawa & Barraclough, 2013; Pons et al., 2006), the Bayesian Poisson Tree Processes (bPTP; (Zhang et al., 2013), and the Automatic Barcode Gap Discovery (ABGD; (Puillandre et al., 2012a). The starting ultrametric Maximum Likelihood tree for GMYC was generated using the chronos function in the ape v. 5.2 package (Paradis & Schliep, 2019) in R. Four different clock models were tested: strict, discrete with ten rate categories, correlated and uncorrelated-relaxed. The best model was selected based on the φ information criterion by Paradis (2013), which takes the penalized term into account. All models were fitted on lambda set to 1.0 and in all cases, the strict clock was found to be the best-fitting model. The single threshold version of GMYC was run on the maximum credibility tree inferred with BEAST and the ultrametric Maximum Likelihood tree in R (v3.5.2) (R Core Team, 2018) using the package splits (Ezard et al., 2009). bPTP analyses were carried out using the bPTP Webserver (http://species.h-its.org/; Zhang et al., 2013) based on the maximum likelihood tree, with 100.000 MCMC generations, sampling every 100 generations, the burn-in set to 0.1 and *T. sylvina* and *D. subpurpurella* were included as the outgroup. For the AGBD analysis, the alignment was submitted to the AGBD online webserver (http://wwwabi.snv.jussieu.fr/public/abgd/; (Puillandre et al., 2012a), with P (prior intraspecific divergence) set from 0.001 to 0.1 and steps set to 10, X (minimum relative gap width) set to 1, Nb bins (from distance distribution) set to 20, selection of the Kimura (K80) model and TS/TV to 2.0.

Life stage associations

Only OTUs for which all three species delimitation approaches yielded the same results (Carstens et al., 2013) were used to further investigate and discuss possible associations between larvae and adults. Moreover, to understand how many of the sampled OTUs are already present

in BOLD, sequences were compared against the BOLD database using BLAST (www.boldsystems.org; Camacho et al., 2009; Ratnasingham & Hebert, 2007). A solid match to a species was assumed when the hit was higher than 99% similarity, at genus level when ≥ 95%, and family ≥ 91% as a rough proxy, following Coddington et al., 2016 and Elbrecht et al., 2017.

Results

Morphotype grouping and phylogenetic inference

The morphological identification of 180 adult and larval specimens resulted in 64 morphospecies from 15 families (see Fig.1). Larval specimens belonged to at least 16 morphospecies from 13 families, whereas adult specimens could be identified up to 47 species from 12 families (Table 1). Brachycentridae, Ecnomidae, Dipseudopsidae, and Polycentropodidae are represented only by larvae, while Helicopsychidae, Calamoceratidae, and Xiphoncentridae are represented only by adult specimens. Furthermore, Brachycentridae, Ecnomidae, and Helicopsychidae were only represented by singletons (Table S1).

Sequence length ranged from 363 to 658 bp with at least 36.5% of identical sites and a GC content of 32.7%. The resulting trimmed COI sequences have been deposited in GenBank. Accession numbers can be found in supplemental Table S1. Gene trees based on ML (Fig. S1) and BI trees (Fig.1) of the COI sequences produced overall similar topologies with just minor differences. Main differences are in the relationships between Ecnomidae and Pseudoneuroclipsidae, Hydroptilidae and Glossosomatidae, and finally Brachycentridae and Goeridae. Morphospecies formed monophyletic entities in almost all cases in the maximum likelihood tree, except for *Glossosoma javanicum* (Glossosomatidae), *Ganonema fuscipenne* (Calamoceratidae), and Leptoceridae (9.2.III). In the Bayesian inferred tree, *G. javanicum* was paraphyletic and *Triplectides indicus* (Leptoceridae) was misplaced as a sister group of Calamoceratidae.

Estimation of species diversity

Species diversity was analyzed based on morphology and three different statistical species delimitation approaches: GMYC, bPTP, and ABGD. Overall, the total number of species yielded by all statistical methods was very similar with 77, 79, and 73, respectively, representing 15 families in total.

Analysis with ABGD generates two different results: the initial and the recursive partition (see Fig. S2 and S3). The recursive partitions usually generate a higher number of clusters; however, the initial partition has proven to give results that best match group assignments of expert taxonomists (Puillandre et al., 2012b). Recursive partitions of the full data set (n= 128 haplotypes) showed that the group assignments range from 1 (P=0.1) to 77 (P= 0.001). On the other side, initial partitions resulted in 73 groups with P-values between 0.001 and 0.0599. The GMYC analysis delimitated 77 OTUs in total (Fig. S4), and the bPTP method recovered 79 OTUs. When applying these three species delimitation methods on our data, six differences could be found: one within Psychomyiidae, three within Hydropsychidae, one within

Lepidostomatidae, and one within Leptoceridae (Table 2). Dissimilarities were caused mainly by AGBD being more conservative in comparison to bPTP and GMYC. Of all families present, Hydropsychidae had an overall much higher species richness with 19 OTUs. Six families were only represented by one OTU each.

Of the 66 OTUs delimited using all three species delimitation approaches, 44 could only be identified to family level in the BOLD database. Only five OTUs (7.6% of all OTUs) could be identified to species level and 4 to genus-level. In 12 cases the OTUs could only be identified as Trichoptera (see green labels in Fig.1). Overall, the morphological identification was consistent with the molecular species diversity delimitation with just four exceptions: *Hydropsyche saranganica* (Hydropsychidae), *G. javanicum*, *Lepidostoma diehli* (Lepidostomatidae), and *G. fuscipenne* which are each divided into two OTUs.

Larval-adult association of Trichoptera

In 17 of the 66 consensus OTUs, representing six families, an association of larval and adult stages was possible (see Table S1). In seven cases, the association involved two or more specimens for each life stage. In ten cases, either the larval or adult stage was represented by only one specimen. It was possible to associate 40 larvae and 38 adults to the following 16 taxa: *Diplectrona gombak*, *Diplectrona pseudofasciata*, *Hydropsyche saranganica*, *Cheumatopsyche globosa/lucida*- complex, *Hydromanicus flavoguttatus*, *Potamyia flavata*, *Chimarra* sp., *Chimarra briseis*, *Agapetus* sp. / *Glossosoma javanicum*-complex, *Lepidostoma diehli/jacobsoni*- complex, *Goera conclusa*, *Adicella* sp., *Oecetis tripunctata*, *Trichosetodes handschini/Setodes musagetes*- complex, *Setodes* sp., and *Rhyacophila* sp.

Discussion

Phylogenetic reconstruction and delimitation methods

The gene trees inferred based on COI of 180 Trichoptera larvae and adults with ML and BI, show very similar topologies. In general, the structure and position of superfamilies and families of both trees reflect the known phylogeny of Trichoptera (Thomas et al., 2020). The main incongruence between ML and BI trees is the unclear relationships between the two genera within Glossosomatidae, *Glossosoma*, and *Agapetus*. COI data suggest that both genera are polyphyletic, as it is the case in several genera of Trichoptera (de Moor & Ivanov, 2008). However, phylogenetic inferences based on single and fast-evolving mitochondrial markers have to be treated with caution, also in Trichoptera. The main goal in larval associations is to find the closest match between adult and larval specimens, in this case with a species delimitation approach on a COI tree (Zhou et al. 2007).

Species delineation and the overall Trichoptera diversity of West Java

To our knowledge, this is the first molecular assessment of the Trichoptera diversity of West Java. A total of 73 to 79 OTUs within 15 Trichoptera families were recovered. In 63 OTUs, the molecular delimitation matched the morphological identification. The only previous study of the

Trichoptera of this area reported 269 species in 12 families near rice fields in the MHSNP (Rizali et al. 2002); the exact information on which species were recorded is however not available. Nonetheless, we can confirm the presence of twelve of the sixteen families reported by Rizali et al. 2002. Moreover, species of six additional families (Calamoceratidae, Dipseudopsidae, Ecnomidae, Goeridae, Lepidostomatidae, Psychomyiidae) are here identified. Different sampling methods and the incorporation of different habitats likely explain the discrepancy.
A comprehensive study on Indonesian Trichoptera diversity (Malicky et al., 2014) reported 146 species from 16 families, based on collections and published data. With the exception of Stenopsychidae, all of the families recorded by Malicky et al. (2014) are also reported here, from one single national park. Moreover, several species recorded here are first records for Java or even undescribed (Mey et al. in prep). In the families Brachycentridae, Lepidostomatidae and Calamoceratidae, nearly all species previously known from Java could be confirmed. The diversity of Hydroptilidae was relatively low compared to previous studies, however; this might be due to the limitations of the net sampling applied here, since specimens of this family are very small and better sampled with Malaise traps (Mey et al. in prep.).
The high number of caddisfly species in this part of West Java suggests the presence of a large range of microhabitats (Dudgeon, 2011). However, due to illegal gold mining and settlements, this habitat diversity is under threat, also within the park. High concentrations and bioaccumulation of heavy metals (e.g. mercury) have already been found in water and sediments (J. Sudarso et al., 2013; Y. Sudarso et al., 2008). These may cause shifts in Trichoptera diversity (Loayza-Muro et al., 2010; Wiederholm, 1984), and lead to morphological abnormalities at least in some species (Yoga et al., 2014a, 2014b).
The application of DNA-barcoding is an efficient method to assess Trichoptera diversity in areas with insufficient or even missing taxonomic knowledge, and can help to assess freshwater stream quality (Sweeney et al., 2011). This is especially valuable, as a clear morphological identification of caddisfly species is in many cases hindered by the absence, or lack of knowledge, of reliable morphological characters (Hjalmarsson et al., 2018). Merely 7.6% of all OTUs identified in this study could be identified to species level when comparing against the BOLD database. Therefore, even though we now have a better idea of the number of species present in the national park, their names and thus the ecological features associated with them remain largely unknown. This underlines the need to expand genetic and morphological studies on Trichoptera in poorly known tropical areas with high diversity, to make DNA-barcoding a more accessible monitoring method of Trichoptera diversity in the Oriental Region, and consequently also a proxy indicator of water quality (Zhou et al., 2016).

Life stage association with DNA-barcoding

DNA-barcoding enabled matching caddisfly larvae and adults in 17 cases. In some of these, only a single individual of one life stage (adults or larvae) was available for inferring the association. Incorporation of higher numbers of both life stages, from a larger number of sites and covering all relevant habitats, would be required for filling the substantial gap remaining; a sound

knowledge of both life stages is in turn essential for understanding inter- and intraspecific variation, ontological variations, and finally the species-specific ecology (Hjalmarsson et al., 2018). While the present study has once more demonstrated the potential of DNA-barcoding to understand and link life stages of Trichoptera, it also highlights the need for a better, more complete barcode reference library of this region. The associated taxa could in most cases be identified to species level as a result of the morphological identification of adult specimens and not by larvae or a match in BOLD. Of the associated species represented by adult and larval specimens in this study, *Pomtayia flavata* was the only species present in BOLD. The collected adult and larval specimens with their correspondent DNA barcodes provide valuable information for future studies in this area.

Conclusions

The present results highlight the potential of DNA-barcoding to identify hidden biodiversity in species-rich and poorly studied taxa. They also show the poor state of exploration of Indonesian Trichoptera, a group of organisms that offers substantial potential as bioindicators for freshwater habitat quality. In Mount Halimun Salak National Park, a protected area of importance for freshwater supply, but nevertheless affected by intensive agriculture and illegal gold mining, freshwater indicators could be of immediate use for monitoring freshwater habitat quality. However, our results show that the inventory of Trichoptera diversity on Java is far from complete, and substantial gaps remain in linking the OTUs uncovered here to species entities. Likewise, substantial work remains to be done in order to link trichopteran life stages. Nevertheless, we would argue that upscaling our approach would, in concert with progress in Trichoptera taxonomy, represent a decisive move towards translating biodiversity data into a monitoring tool of wide applicability.

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Figure 1

Bayesian inferred ultrametric phylogenetic tree based on COI of 182 Trichoptera larvae (in bold) and adults.

Bootstrap values higher than 0.5 are indicated above branches. Results of the three different species delimitation approaches (bPTP, GMYC, and AGBD) are illustrated by vertical bars. Each bar represents an Operational Taxonomic Unit (OTU) detected by the respective approach labeled on the top. Morphological identification of adult (in bold) and ~~juvenile~~ specimens were added directly on the leaves. Green tips are species also identified in BOLD following the thresholds by Elbrecht et al. 2017. OTUs endorsed by all the delimitation methods are numbered in the first column and BLAST results are summarized in Table 1. Additionally, confirmed OTUs with juvenile and adult specimens are marked with a symbol at the end of the vertical bars. N= number of OTU´s collapsed in the phylogenetic tree, n= number of haplotypes collapsed to one sequence.

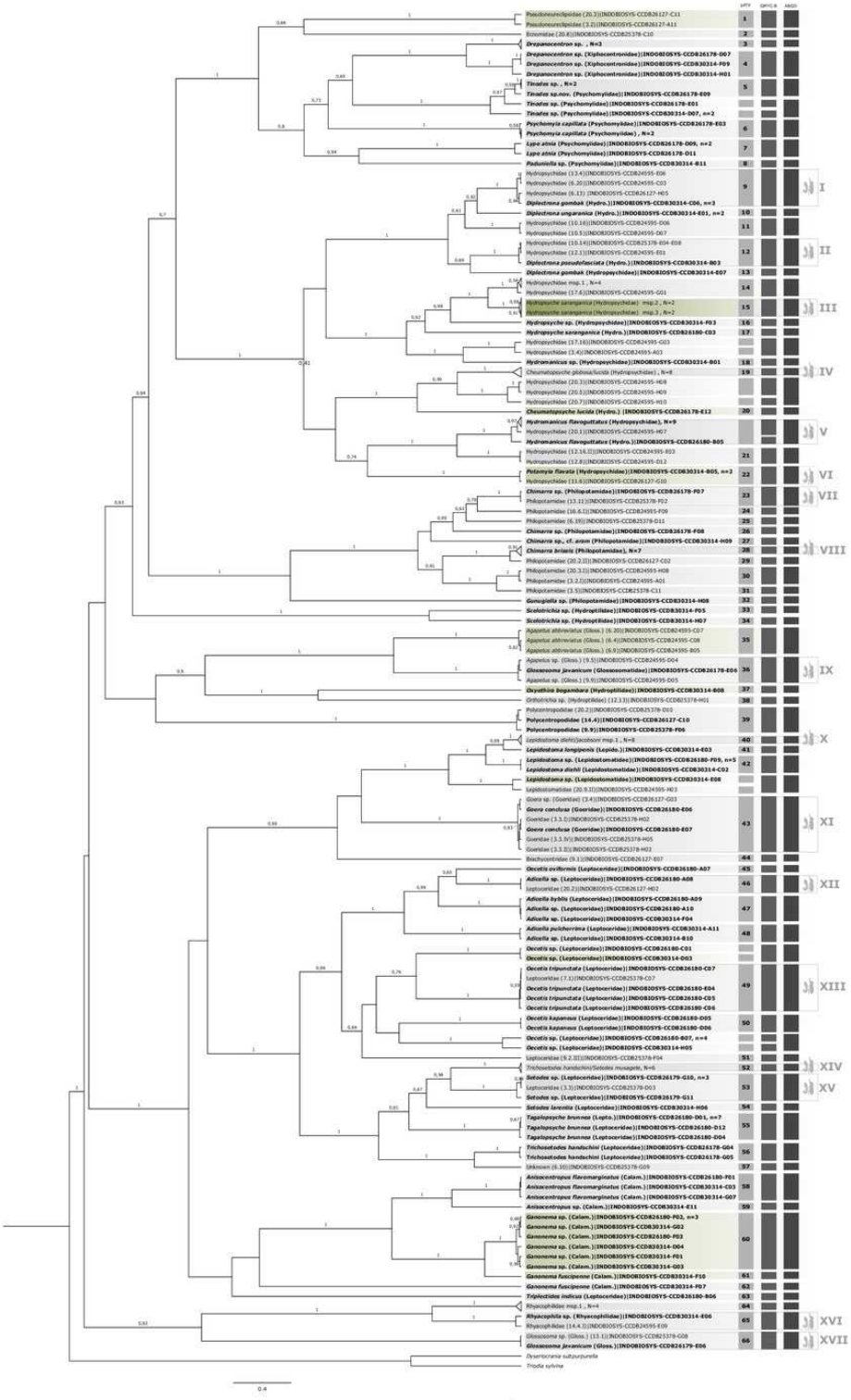

Table 1(on next page)

Trichoptera diversity in and around the Mount Halimun Salak National Park, West Java, derived from adult and larval records (no. of specimens).

Family	Taxa	Larva	Adult
Brachycentridae		1	
Calamoceratidae	*Anisocentropus flavomarginatus* Ulmer, 1906		3
Calamoceratidae	*Anisocentropus* sp.		2
Calamoceratidae	*Ganonema fuscipenne* Albarda, 1881		4
Calamoceratidae	*Ganonema ochraceellum* McLachlan, 1866		2
Pseudoneureclipsidae		2	
Ecnomidae		1	
Glossosomatidae	*Glossosoma javanicum* Ulmer, 1930		2
Glossosomatidae	*Glossosoma* sp.	1	
Goeridae	*Goera conclusa* Ulmer, 1905		2
Goeridae	*Goera* sp.	1	
Goeridae		3	
Helicopsychidae	*Helicopsyche* sp.		1
Hydropsychidae	*Agapetus* sp.	5	
Hydropsychidae	*Cheumatopsyche globosa* Ulmer, 1910		1
Hydropsychidae	*Cheumatopsyche lucida* Ulmer, 1907		2
Hydropsychidae	*Cheumatopsyche* sp.		2
Hydropsychidae	*Diplectrona gombak* Olah, 1993		2
Hydropsychidae	*Diplectrona pseudofasciata* Ulmer, 1909		1
Hydropsychidae	*Diplectrona ungaranica* Ulmer, 1951		1
Hydropsychidae	*Hydromanicus flavoguttatus* Albarda, 1881		4
Hydropsychidae	*Hydromanicus* sp.		2
Hydropsychidae	*Hydropsyche saranganica* Ulmer, 1951		3
Hydropsychidae	*Hydropsyche* sp.		1
Hydropsychidae		32	2
Hydroptilidae	*Orthotrichia* sp.	1	
Hydroptilidae	*Scelotrichia* sp.		2
Hydroptilidae			1
Lepidostomatidae	*Lepidostoma diehli* Weaver, 1989		2
Lepidostomatidae	*Lepidostoma jacobsoni* Ulmer, 1910		1
Lepidostomatidae	*Lepidostoma longipenis* Weaver, 1989		1
Lepidostomatidae	*Lepidostoma* sp.		4
Lepidostomatidae		5	
Leptoceridae	*Adicella byblis* Malicky, 1998		1
Leptoceridae	*Adicella pulcherrima* Ulmer, 1906		1
Leptoceridae	*Adicella* sp.		4
Leptoceridae	*Oecetis kapaneus* Malicky, 2005		2
Leptoceridae	*Oecetis oviformis* Ulmer, 1951		1
Leptoceridae	*Oecetis* sp.		4
Leptoceridae	*Oecetis tripunctata* Fabricius, 1793		4
Leptoceridae	*Setodes larentia* Malicky & Chantaramongkol, 2006		1
Leptoceridae	*Setodes musagetes* Malicky & Chantaramongkol, 2006		2
Leptoceridae	*Setodes* sp.		2
Leptoceridae	*Tagalopsyche brunnea* Ulmer, 1905		3

Leptoceridae	*Triplectides indicus* Walker, 1852		1
Leptoceridae	*Trichosetodes handschini* Ulmer, 1951		3
Leptoceridae		7	
Philopotamidae	*Chimarra briseis* Malicky, 1998		3
Philopotamidae	*Chimarra* sp.		3
Philopotamidae	*Chimarra* sp., cf. *aram*		1
Philopotamidae	*Gunugiella* sp.		1
Philopotamidae		10	
Polycentropodidae		3	
Psychomyiidae	*Lype atnia* Malicky & Chantaramongkol, 1993		2
Psychomyiidae	*Paduniella* sp.		1
Psychomyiidae	*Psychomyia capillata* Ulmer, 1910		2
Psychomyiidae	*Psychomyia* sp.		
Psychomyiidae	*Tinodes* sp.		4
Psychomyiidae	*Tinodes* sp.nov.		1
Rhyacophilidae	*Rhyacophila* sp.		1
Rhyacophilidae		5	
Xiphocentronidae	*Drepanocentron* sp.		3
Xiphocentronidae	*Drepanocentron* sp.1		2
Xiphocentronidae	*Drepanocentron* sp.2		1
Unknown		1	
Total		**78**	**102**

1

Table 2(on next page)

Total number of OTUs per family based on the different species delimitation approaches.

Family	bPTP	GMYC	ABGD
Brachycentridae	1	1	1
Calamoceratidae	4	4	4
Dispeudopsidae	1	1	1
Ecnomidae	1	1	1
Glossosomatidae	3	3	3
Goeridae	1	1	1
Hydropsychidae	19	19	17
Hydroptilidae	1	1	1
Lepidostomatidae	5	4	4
Leptoceridae	11	10	9
Polycentropodidae	1	1	1
Philopotamidae	9	9	9
Psychomyiidae	6	6	5
Rhyacophilidae	2	2	2
Xiphocentronidae	2	2	2
Others	12	12	12
Total	**79**	**77**	**73**

1