Treubia, 49(2): 97–114, December 2022 DOI: 10.14203/treubia.v49i2.4402 https://e-journal.biologi.lipi.go.id/index.php/treubia

THE DISCOVERY OF CHALCORANA MEGALONESA IN JAVA AND THE EVALUATION OF ITS TAXONOMIC STATUS

Efendi Sabinhaliduna¹, Tuty Arisuryanti¹, and Amir Hamidy²*

¹Graduate Program, Faculty of Biology, Universitas Gadjah Mada,
Jl.Teknika Selatan, Sleman, Yogyakarta, Indonesia

²Laboratory of Herpetology, Museum Zoologicum Bogoriense, Research Center for Biosystematics and
Evolution, National Research and Innovation Agency (BRIN),
Gd. Widyasatwaloka, Jl. Raya Jakarta-Bogor Km 46, Cibinong, West Java, Indonesia

*Corresponding author: hamidyamir@gmail.com; amir006@brin.go.id

Received: 10 September 2022; Accepted: 23 December 2022; Published: 30 December 2022

ABSTRACT

Chalcorana chalconota was firstly described as Hyla chalconotus Schlegel from Java, and widely recognized as Rana chalconota Boulenger. This ranid frog species has widespread distribution in Southeast Asia. Molecular studies revealed that Rana chalconota is a complex species and consisted of several cryptic species. Among Sundaland population, this group was split into five species (C. chalconota sensu stricto, C. parvaccola, C. rufipes, C. raniceps, C. megalonesa), and together with C. labialis and C. eschatia, they nested in a monophyletic group. During herpetological survey in West Java, we collected two morphotypes of Rana chalconota from Pasir Angin, Bogor and evaluated their taxonomy status using morphology approach and molecular data with 16S rRNA mitochondrial gene. The results revealed two morphotypes belong to C. chalconota sensu stricto and C. megalonesa. The genetic distance of C. megalonesa between Java population and Sarawak (Borneo) population ranged from 2.4 to 2.6% that still below the threshold of species delimitation on the 16S rRNA gene. Therefore, these two populations are conspecific. This discovery of C. megalonesa in Java has contributed to increase the number of species of amphibians in Java to become 46 species.

Key words: Chalcorana megalonesa, Java, new record, taxonomy

INTRODUCTION

Chalcorana chalconota was first described as Hyla chalconotus (Schlegel, 1837) based on the type series collected from Java and Sumatra. The species is widely known as Rana chalconota (Boulenger, 1882) and is widespread in Southeast Asia. This ranid frog breeds along streams in various lowland forests, from primary and secondary forests to swamp forests (Inger, 1969). Rana chalconota is a slender species, small to moderate-sized species (adults 30–60 mm snout-vent length). The tips of the digits, especially of the fingers, are distinctly enlarged and have circummarginal grooves. The toe webbing is extensive, reaching the distal subarticular tubercle of the fourth toe or slightly beyond. The general coloration is green above and white or cream-coloured below and the upper lip is usually distinctly lighter than the adjacent areas (Inger et al., 2009). The molecular studies proved that a widely distributed species in fact represent several cryptic species. This phenomenon also occurs in the species Rhacophorus dugritei (Li et al., 2012), Polypedates leucomystax (Kuraishi et al., 2013), Leptobrachium hasseltii (Hamidy & Matsui, 2017), and the Microhyla complex (Atmadja et al., 2018).

Based on morphological and molecular studies, (Inger et al., 2009) divided the *C. chalconota* into several different species using collection specimens. The population found in Kalimantan consisted of *C. megalonesa* and *C. raniceps*; *C. parvaccola* and *C. rufipes* in



Sumatra; and *C. chalconota* in southern Sumatra and Java. In addition, the Malay Peninsula population is *C. labialis*, and in Thailand it is *C. eschatia*, and both of them are nested in a monophyletic group of *C. chalconota* group. However, this comprehensive study still lacks information on morphological characters from the fresh specimens.

Over the past few years, various fieldwork had helped increase documentation of the distribution of this frog group. Our recent survey revealed that two morphotypes *C. chalconota* co-exist in one place in Pasir Angin, Bogor. Therefore, in this study we evaluate the taxonomy status of the two morphotypes *C. chalconota* group based on molecular analysis and morphological data from fresh specimen. Morphological study from fresh specimens might identify a number of *characters* that have, in the past, been misinterpreted.

MATERIALS AND METHODS

Sampling

Samples were collected from Wikasatrian Leadership Center Forest, Pasir Angin, Gadog, Bogor, West Java. A total of 12 specimens of *C. chalconota* group were brought to the laboratory for morphological and molecular analysis. The specimens were fixed in 10% formalin and then preserved in 70% ethanol and stored in the Museum Zoologicum Bogoriense (MZB) as specimen vouchers (MZB Amph 32567-32577, MZB Amph 32580) (Fig. 1).

DNA extraction

Total genomic DNA was extracted from a small amount of frozen muscle or liver tissue from four frog samples. We amplified and sequenced partial 16S mitochondrial rRNA (16S) gene fragments of ca. 394 bp using the primers H3056 5'-CTC CGG TCT GAA CTC AGA TCA CGT AGG-3'; L2606 5'-CTG ACC GTG CAA AGG TAG CGT AAT CACT-3' (Hedges et al., 1993). Detailed protocols, reagents, and polymerase chain reaction (PCR) conditions followed Hamidy et al. (2018) and Munir et al. (2018). The four new sequences were deposited in GenBank with the accession numbers OQ080017, OQ080018, OQ080019, OQ080020 (Table 1).

Phylogenetic Analyses

In this study, sequences were assembled and checked manually using Chromas Pro software (Technelysium Pty Ltd., Brisbane, Australia) to obtain consensus sequences. The results of consensus sequences were carried out on each individual using forward and reverse primers. The chromatogram obtained was then manually edited to see ambiguous nucleotide bases and stop codons. Furthermore, for the reconstruction of phylogenetic trees, nucleotide sequences were first aligned with genbank sequences using MUSCLE in the MEGAXI (Tamura et al., 2021). Reconstruction of the phylogeny tree using Neighbor-Joining (NJ), Maximum Likelihood (ML,) and Bayesian Inferences (BI). NJ and ML analysis used the MEGAXI with 1000 replications, Tamura-3 parameter model with transversions-transition substitution, and gaps complete deletion. DNA evolution model for BI using J2_gamma model selected using Kakusan 3 and BI analysis were conducted using Metropolis coupled Markov chain Monte Carlo chains (MCMC) for 10.000.000 generations, sampled every 1.000 generations and discarding the first 25% trees as burn-in, using MrBayes v3.2.7 (Ronquist & Huelsenbeck, 2003), Run convergence was evaluated at a split frequency of <0.01 standard deviations. The tree consensus is visualized in FigTree 1.4.4 (Rambaut, 2019).

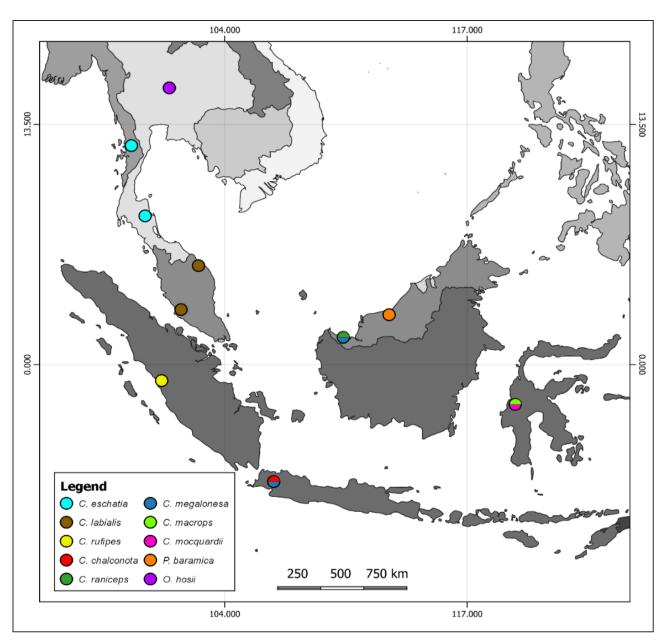


Figure 1. Map of Southeast Asia showing sample localities of Chalcorana group dan outgroup included in this study.

Tabel 1: Samples used for 16S rRNA analysis in this study, together with species identification, specimen voucher number, locality, GenBank accession numbers, and references.

Species	Voucher	Location	GenBank	Sumber
C. chalconota	MZB Amph 32569	Indonesia, West Java, Bogor	0Q080018	This study
C. chalconota	MZB Amph 32580	Indonesia, West Java, Bogor	0Q080017	This study
C. megalonesa	MZB Amph 32576	Indonesia, West Java, Bogor	OQ080020	This study
C. megalonesa	MZB Amph 32577	Indonesia, West Java, Bogor	OQ080019	This study
C. megalonesa	RZ 119	Malaysia, Bomeo,Sarawak	DQ835325	Zainudin et al., 2017
C. megalonesa	RZ 173	Malaysia, Borneo, Sarawak	DQ835326	Zainudin et al., 2017
C. megalonesa	FMNH 268981	Malaysia, Borneo, Sarawak	KR264085	Oliver et al., 2015
C. raniceps	BNP014	Malaysia, Borneo,Sarawak	DQ835322	Zainudin et al., 2017
C. raniceps	BNP037	Malaysia, Borneo, Sarawak	DQ835323	Zainudin et al., 2017
C. rufipes	FMNH 268575	Indonesia, West Sumatra, Padang	KR264081	Stuart et al., 2006
C. rufipes	MVZ 239431	Indonesia, Sumatra.	KR264095	Oliver et al., 2015
C. labialis	852	Peninsular Malaysia, Bukit Gasing	KF738962	Awang, 2013
C. labialis	853	Peninsular Malaysia, Bukit Gasing	KF738963	Awang, 2013
C. labialis	1326	Malaysia, Terengganu, Kampung Jabi, Lata Belatan	KF738985	Awang, 2013
C. labialis	1327	Malaysia, Terengganu, Kampung Jabi, Lata Belatan	KF738986	Awang, 2013
C. eschatia	FMNH 268851	Thailand, Khao Luang National Park	KR264083	Oliver et al., 2015
C. eschatia	FMNH 268859	Thailand, Khao Luang Bencha Park	KR264084	Oliver et al., 2015
C. eschatia	USNM Herp 586971	Tanintharyi Myanmar to southern Thailand	MG935754	Mulcahy et al., 2018
C. eschatia	USNM Herp 586978	Tanintharyi Myanmar to southern Thailand	MG935755	Mulcahy et al., 2018
C. mocquardii	MVZ 254779	Indonesia, Sulawesi	KR264099	Oliver et al. 2015
C. mocquardii	MVZ 254762	Indonesia, Sulawesi	KR264100	Oliver et al., 2015
C. macrops	MVZ 254478	Indonesia, Sulawesi	KR264098	Oliver et al., 2015
Pulchrana baramica	FMNH 266574	Malaysia, Bukit Sarang	AY994204	Inger, 2006
Oddorana hosii	FMNH 268250	Thailand	DQ650602	Stuart et al., 2006

Morphology Analyses

We examined all specimens labelled *C. chalconota* Java and Borneo, stored in the Laboratory of Herpetology, Museum Zoologicum Bogoriense, Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN) (Appendix). During examination we re-indentified the specimens and categorized as *C. chalconota* and *C. megalonesa* based on the morphological characters. We measured 18 characters with a dial caliper to the nearest 0.1 mm. The following characters were taken from (Inger et al., 2009) and (Matsui & Maeda, 1999): Snout-vent length (SVL), Head length (HL), Head width (HW), Tympanium diameter (TD), Tibia length (TL) Eye diameter (ED)), Hand and arm length (HAL), Upper eyelid length (UEL), Upper eyelid width (UEW), Snout length (SL), Internarial distance (ID), Distance between anterior tips of upper eyelids (DIE), Interorbital width (IW), Distance between norsil and eye N (EL), Third finger length (3FL), Disc of the third finger (DF3), Fourth toot length (TOEL4), and Disc of the fourth toot (DT4).

RESULTS

Phylogenetic Relationship

The sequence statistic for the partial gene fragment obtained from 394 nucleotide positions consisted of 267 conserved sites, 110 variable sites, 90 parsimony-informative sites, and 20 single sites. Neighbor Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses resulted in essentially the same topologies, they differ only in poorly supported nodes. The NJ, ML, BI tree (Fig. 2) and genetic distance (Table 2) infer the following sets of relationships:

- 1. The genus of *Chalcorana* is monophyletic with a high bootstrap values (NJ = 99%, ML = 99%, BI = 1.00%) with respect to *Pulchrana baramica* and *Odorrana hosii* as the outgroups.
- 2. This monophyletic group consists of two clades. Clade A represents *C. chalconota* group from Peninsular Malaysia, Thailand, Myanmar, Borneo, Java, and Sumatra; (NJ = 89%, ML = 89%, BI = 1.00%) while clade B is *C. mocquardii* from Sulawesi (NJ=100%, ML=100%, BI=1.00%).
- 3. Clade A consists of subclades paraphyletic I, II, and III.
- 4. Subclade I consists of *C. labialis* from Peninsular Malaysia, *C. eschatia* from Thailand and Myanmar, *C. megalonesa* from Java and Sarawak Malaysia, (NJ=99%, Ml=99%, BI=1.00%). Subclade II consists of *C. chalconota* from Java which is a sister taxon with *C. rufipes* from West Sumatra, with highly supported values (NJ=87%, ML=89%, BI=0.99%). Furthermore, subclade III consists of *C. raniceps* from Borneo, with highly supported (NJ=100%, ML=100%, BI=1.00%).

Table 2. Genetic distance (p-distance) Chalcorana genera group and outgroup in the study

		2	8	4	v	9	7	∞	6	10 11	=	12
1. C. chalconota Java	0.0											
2. C. megalonesa Java	9.5	0.0										
3. C. megalonesa Sarawak	8.6-9.2	2.4-2.6	0.8-1									
4. <i>C. Iabialis</i> Peninsular Malaysia	6.8-7.4	4-4.5	3.4-4.6	8.0-0.0								
5. C. eschatia Thailand	7.1	4.3	3.4-4.6	1.6-2.9	0.0							
6. C. eschatia Myanmar	7.4	4	3.2-3.4	2.1-3.2	8.0	0.0						
7. C. raniceps Sarawak	6.3	8.9	9.8-8	7.1	9.9	8.9	0.0					
8. C. rufipes Sumatera Barat	4.9	6.6	9.2-9.3	8.4-8.7	8.7	8.4	7.5	0.0				
9. C. mocquardii Sulawesi	11.6	11.9-12.5	11-12	11.6-12.2	12.5	12.8	10.7-11	11.9-12	4			
10. C. macrops Sulawesi	18.8	18.9	17.9-18.2	17.2	17	16.9	16	18.9	15.1-16.4	0.0		
11. Oddorana hosii Thailand	21.7	20.7	19.8-20.8	21.2-21.6	20.3	20.8	22	20.1	19.8-21	18.6	0.0	
12. <i>Pulchrana baramica</i> Malaysia	22.5	23.4	23.6-24.7	23.7-24.2	22.9	22.7	23.5	22.8	23.9-26.9	21.2	16.1	0.0

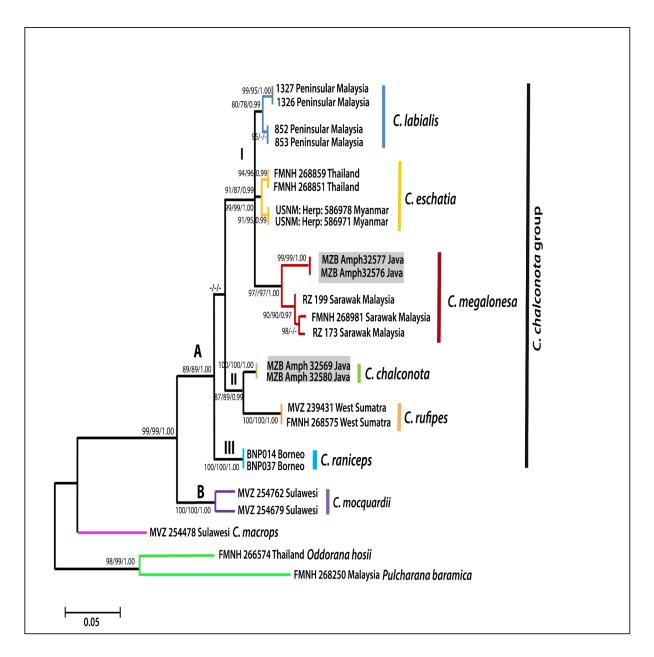


Figure 2. Phylogenetic tree of 16S rRNA in *Chalcorana* group. Values in the branch indicate the NJ, ML, and BI bootstrap. The scale in the topology represents the genetic value of each branch.

Taxonomy Chalcorana chalconota (Schlegel, 1837)

Specimen MZB Amph 32567, 32568, and 32580 are three adult females. MZB Amph 32569, 32570, and 32572 three adult males collected, from Wikasatrian, Pasir Angin, Cipayung Datar, Megamendung District, West Java Province by Amir Hamidy, Huda Wiradarma, Quraisy Zakky and Syaripudin (Figs 3, 4, 5).

Description. SVL in males up to 37.3-48.9 mm, females up to 52.6-70.4 mm (Table 3). Head triangular and slightly wider; snout sloping; the skin texture on the dorsal part has small protrusions so looks rougher; there are white spots on the lateral body; the dorsolateral fold visible, webbing on the hind limbs is red, and toe webbing formula 1(0), 2(0/0), 3(0.5/0), 4(0.5/0,5), 5(0).

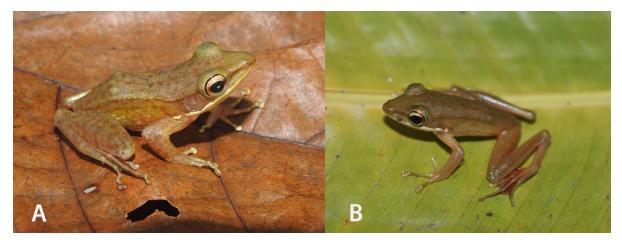


Figure 3. Life specimen of *Chalcorana chalconota* from Java. (A) female, MZB Amph 32580 and (B) male, MZB Amph 32569.



Figure 4. Specimen of *Chalcorana chalconota* from Java. (A) female, MZB Amph 32580, and (B) male, MZB Amph 32569. Dorsal view (first row), ventral view (second row), lateral view (third row), and dorsal sides of thigh (fourth row). Scale bar 10 mm.



Figure 5. Specimen of *Chalcorana chalconota* from Java. (A) female, MZB Amph 32580 and (B) male, MZB Amph 32569. Hand (top row) and foot (bottom row). Scale bar 5 mm.

Chalcorana megalonesa (Inger et al., 2009)

Specimen. MZB Amph 32571, 32575, and 32576 are three adult females. MZB Amph 32573, 32574, and 32577 three adult males. The specimens collected, from Wikasatrian, Pasir Angin, Cipayung Datar, Megamendung District, West Java Province by Amir Hamidy, Huda Wiradarma, Quraisy Zakky and Syaripudin (Figs. 6, 7, 8, 9).

Description. SVL in males up to 26.1-30.4 mm, females 35.7-45.4 mm (Table 3). Head triangular, slightly smaller; snout protruding; a bright green color on the lateral part of the body; the skin texture on the dorsal part is smoother; the dorsolateral fold not clearly visible, the web on the hind limbs is black, and toe webbing formula 1(0), 2(1/0), 3(1/0), 4(1,5/1), 5(0).

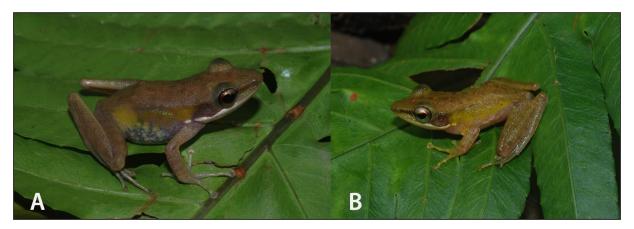


Figure 6. Chalcorana megalonesa of life specimen from Java. (A) female, MZB Amph 32575 and (B) male, MZB Amph 32577.

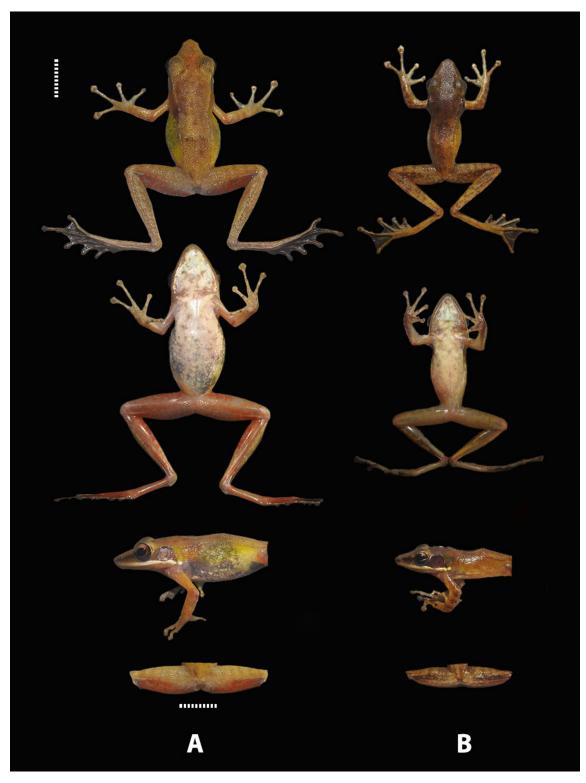


Figure 7. Specimen of *Chalcorana megalonesa* from Java. (A) female, MZB Amph 32575 and (B) male, MZB Amph 32577. Dorsal view (first row), ventral view (second row), lateral view (third row), and dorsal sides of thigh (fourth row). Scale bar 10 mm.



Figure 8. Specimen of *Chalcorana megalonesa* from Java. (A) female, MZB Amph 32575 and (B) male, MZB Amph 32577. Hand (top row) and foot (bottom row). Scale bar 5mm.

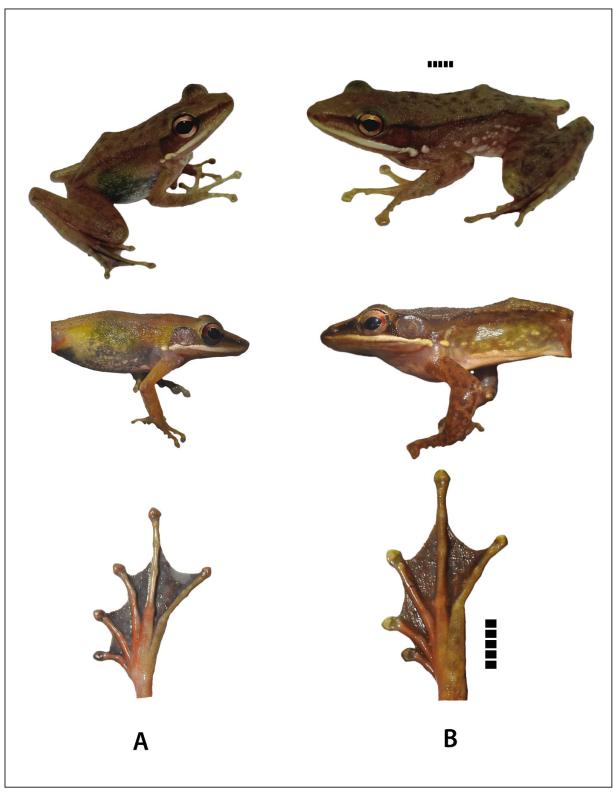


Figure 9. Two species of the *Chlacorana chalconota* groups in life from Java. (A) a Female of *C. megalonesa* (MZB Amph 32576) and (B) a mature female specimen of *C. chalconota* (MZB Amph 25680). of life specimens view (top row), lateral view (middle row) and foot in dorsal view (bottom row), from Java. Scale bar = 5

Table 3. Morphometric variation of selected measurement (mm) in C. chalconota and C. megalonesa, from Java and Borneo

	C chalconota	Cchalconota	C megalonesa	Cmegalonesa	C megalonesa	Cmegalonesa
Character (mm)	male $(n = 10)$	female (n=10)	male $(n = 10)$	female (n = 10)	male (n = 10)	female $(n = 10)$
	Location Java	Location Java	Location Java	Location Java	Location Borneo	Location Borneo
SVL	37.3-48.9	52.6-70.4	26.1-30.4	35.7-45.4	31.3-43.3	45.5-62.3
HL	14.4-19.8	20.1-26.7	10.9-11.8	13.0-17.0	12.6-18.7	16.3-24.7
HW	11.6-16.3	16.4-21.8	9.6-6.7	10.4-13.9	9.7-13.9	13.2-20.4
TD	3.5-4.8	4.3-6.3	3.0-3.7	3.5-4.0	3.1-4.9	4-4.9
TL	22.1-29.9	30.4-41.8	14.8-16.8	16.5-27.8	19.2-25.6	24.4-36.8
ЕD	3.3-4.6	4.4-6.6	2.4-2.9	2.7-3.9	2.6-4.9	3.7-5.6
HAL	18.9-26.4	25.4-33.6	13.4-15.9	17.7-24.1	15.7-23.7	21.4-32.9
UEL	5.2-7.1	6.4-8.9	4.4-4.7	4.8-6.7	4.7-6.8	5.6-8.5
UEW	3.1-4.3	4.01-5.84	2.4-2.6	2.7-3.5	2.5-3.9	3.1-5.7
SL	6.4-8.7	8.9-12.3	4.2-4.9	5.5-7.8	5.4-6.9	6.5-9.8
ID	3.2-3.9	4.2-6.0	2.2-2.8	3.3-3.8	3.2-3.9	3.7-5.1
DIE	6.2-8.9	8.6-11.8	4.1-5.3	5.7-8.0	5.9-7.9	7.4-11
IW	3.4-4.6	4.7-6.8	2.6-3.1	3.2-4.4	3.2-4.5	4.1-6.8
N EL	4.0-5.4	5.7-7.6	2.8-3.3	3.3-5.1	3.6-4.9	4.6-7.5
3FL	10.4-15.7	15.2-20.7	8.6-6.7	11.3-14.0	9.1-13.4	12.1-19.4
DF3	1.4-2.1	2.2-3.6	1.0-1.4	1.1-2.4	1-2.7	1.9-3.8
TOEL4	18.6-27.5	27.2-36.6	13.6-15.9	17.8-25.4	14.9-22.6	20.9-29.8
DT4	0.9-1.7	1.3-2.6	0.6-1.0	0.7-1.7	0.7-1.2	1.1-2.1

DISCUSSION

Chalcorana megalonesa was distributed in Borneo and was first described based on specimen collections found in Bukit Sarang, Bintulu, Sarawak. The name of this species is taken from "megalo" which means island, and "nesos" which means big, these refer to its distribution on the large island of Borneo (Inger et al., 2009). Our study revealed the occurrence of this species in Java Island. Moreover, this species co-exist in the same habitat with C. chalconota in areas of Wikasatrian Leadership Center Forest, Pasir Angin, Gadog, Bogor, West Java whereas the habitat type is wetland with Kirai Forest (Kirai is Metroxylon sagu Rottb). Other amphibian species that also inhabit this habitat type are Pulchrana baramica Bottger, 1900; Limnonectes macrodon Dumeril and Bibron, 1841; Polypedates leucomystax Gravenhorst, 1829; Rhacophorus rendwardtii Schlegel, 1840; Ferjevarya limnocharis Gravenhorst, 1829; and Microhyla achatina Tschudi, 1838.

The discovery of the species *C. megalonesa* provides a new record of increasing the number of amphibian species on the island of Java which was previously reported as many as 45 species (Alhadi et al., 2021) to 46 species. Morphologically, both *C. chalconota* and *C. megalonesa* from Java are distinct and possible to be distinguished morphologically. Some of the characteristics can be used are Snout-vent length (SVL), the shape of the snout, the presence or absence o the dorsolateral folds, the color and pattern on the laterals, the color of the webbing on the hind limbs (fresh specimens), as well as the webbing formula (Figs 4, 5, 7, 8, 9).

The results of the phylogenetic reconstruction analysis of the *C. chalconota* group Java are depicted on a phylogenetic tree (Fig. 2). The *C. megalonesa* specimens collected in Java (MZB Amph 32576-32577) are on one clade with *C. megalonesa* from Borneo (RZ 119, RZ 173, and FMNH 268981). Furthermore, the genetic distance between the Java and Borneo groups (2.4-2.6%), indicates that these groups are conspecific. According to Fouquet et al. (2007), the interspecific threshold of genetic distance based on the 16S rRNA gene in amphibians is 3%. Although, *C. parvaccola* is not included in the phylogenetic analysis study because of the incompatibility of sequence data obtained from the genebank, the phylogenetic studies by Inger et al. (2009) confidently placed *C. parvaccola* as a distinct monophyletic group separated from as *C. chalconota* and *C. megalonesa*.

Intraspecific genetic variation of *C. megalonesa* from Java and Borneo (Sarawak) ranged from 2.4-2.6 % and concordant with a morphological variation whereas population from Java generally smaller [males <30.4 mm, females <45.4 mm Vs males >35 mm and females >50 mm (Inger et al. 2009)]. However, this morphological variation needs further investigation by involving more samples to get comprehensive conclusion.

The discovery of *C. megalonesa* in Java is not only restricted in West Java, but also include other population in Central Java and East Java (Fig. 10). This indicates distribution of this species is larger than expected so far. Further studies by including larger area sampling, including Sumatra are needed to comfirm the distribution of this species that can be useful to evaluate its IUCN redlist status.



Figure 10. Localities new records of Chalcorana megalonesa on the island of Java.

ACKNOWLEDGMENTS

We thank PT Wijaya Karya (Wikasatrian Leadership Center) for permits to conduct the biodiversity inventory, Tonny Warsono, Tamjianto, Prof. Dedy Darnaedi for supports during biodiversity inventory. We thank the reviewers for all the input and suggestions in improving the manuscript. We express our gratitude and condolence to the late young talented herpetologist, Farists Alhadi who promoted Indonesian wildlife photography and Indonesian herpetological surveys during his life. We also thank Misbahul Munir for his discussion, Tri. S. Rachman, Huda Wiradarma, Syaripudin, Fajar Kaprawi and Quraisy Zakky for their assistantships during the fieldwork and we also thank Mulyadi and Wahyu Trilaksono (the technicians at the Laboratory of Herpetology, Museum Zoologicum Bogoriense, Research Center for Biosystematics and Evolution, National Research and Innovvation Agency (BRIN)), for helping a lot in providing research needs in the examination of specimen collections. All authors are main contributors and contributed equally to the manuscript.

REFERENCES

Alhadi, F., Kaprawi, F., Hamidy, A. & Kirschey, T. 2021. *Panduan Bergambar dan Identifikasi Amfibi Pulau Jawa*. Perkumpulan Amfibi Reptil Sumatra (ARS/NABU). Jakarta.

Atmadja, V.Y., Hamidy, A., Arisuryanti, T., Matsui, M. & Smith, E.N. 2018. A new species of *Microhyla* (Anura: Microhylidae) from Sumatra, Indonesia. *Treubia*, 45: 25–46.

Awang, Z.B. 2013. Filogeni Katak Ranidae di Semenanjung Malaysia Berdasarkan Morfologi dan Gen 16S rRNA Mitokondria. [Tesis]. Universiti Sains Malaysia. Malaysia.

Boulenger, G.A. 1882. Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum. London: Taylor & Francis.

Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. 2007. Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE*, 1–8.

Hamidy, A. & Matsui, M. 2017. Phylogenetic relationship of *Leptobrachium hasseltii* Tschudi, 1838 (Amphibia, Anura, Megophrydae) Detection of a possible cryptic species. *Treubia*, 44: 15–28.

- Hamidy, A., Munir, M., Mumpuni, M., Rahmania, M. & Kholik, A.A. 2018. Detection of cryptic taxa in the genus Leptophryne (Fitzinger, 1843) (Amphibia: Bufonidae) and the description of a new species from Java, Indonesia. *Zootaxa*, 4450(4): 427–444.
- Hedges, S.B., Nussbaum, R.A. & Maxson, L.R. 1993. Caecilian phylogeny and biogeography inferred from mitochondrial DNA sequences of the 12 rRNA and 1g rRNA genes (Amphibia, Gmnophiona). *Herpetological Monographs*, 7: 64–76.
- Inger, R.F. 1969. Organization of communities of frogs along small rain forest streams in Sarawak. *Journal of Animal Ecology*, 38: 123–148.
- Inger, R.F., Stuebing, R.B. & Stuart, B.L. 2006. The Tadpole of *Rana glandulosa* Boulenger (Anura: Ranidae). *The Raffles Bulletin of Zoology*, 54(2): 465–467.
- Inger, R.F., Stuart, B. L., & Iskandar, D. T. 2009. Systematics of a widespread Southeast Asian frog, *Rana chalconota* (Amphibia: Anura: Ranidae). *Zoological Journal of the Linnean Society*, 155:123–147.
- Kuraishi, N., Matsui, M., Hamidy, A., Belabut, D.M., Ahmad, N., Panha, S., Sudin, A., Yong., H.S., Jiang, J.P. & Ota, H. 2013. Phylogenetic and taxonomic relationship of the *Polypedates leucomystax* complex (Amphibia). *Zool Script*, 42:54–70.
- Li, J.T., Liu, J., Chen, Y.Y., Wu. J.W., Murphy, R.W., Zhao, E.M., Wang, Y.Z. & Zhang, Y.P. 2012. Molecular phylogeny of treefrogs in the *Rhacophorus dugritei* species complex (Anura: Rhacophoridae), with descriptions of two new species. *Zoological Journal of the Linnean Society*, 165: 143–162.
- Matsui, M. & Maeda, N. 1999. *Frogs and Toads of Japan*. Tokyo (Japan): Kyoto University Press: pp.10–11.
- Munir, M., Hamidy, A., Farajallah, A. & Smith, E.N. 2018. A new *Megophrys* Kuhl and van Hasselt (Amphibia: Megophryidae) from southwestern Sumatra, Indonesia. *Zootaxa*, 4442 (3): 389–412.
- Mulcahy, D.G., Lee, J.I., Miller, A.H., Chand, M., Thura, M.K. & Zug, G.R. 2018. Filling the BINs of life: Report of an amphibian and reptile survey of the Tanintharyi (Tenasserim) Region of Myanmar, with DNA barcode data. *ZooKeys*, 757: 85–152.
- Oliver, L.A., Prendini, E., Kraus, F. & Raxworthy, C.J. 2015. Systematics and biogeography of the Hylarana frog (Anura: Ranidae) radiation across tropical Australasia, Southeast Asia, and Africa. *Molecular Phylogenetics and Evolution*, 90: 176–192.
- Rambaut, A. 2019. FigTree v 1.4.4. [Online]http://tree.bio.ed.ac.uk/software/figtree/ [Accessed 22 April 2022].
- Ronquist, F. & Huelsenbeck, JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.
- Schlegel, H. 1837. Abbildungen neuer oder unvollständig bekannter Amphibien, nach der Natur oder dem Leben entworfen, herausgegeben und mit einem erläuternden Texte begleitet Düsseldorf: Museum of Comparative Zoology: German: pp. 23–25.
- Stuart, B. L., Inger, R.F., & Voris, H. K. 2006. High level of cryptic species diversity was revealed by sympatric lineages of Southeast Asian forest frogs. *Biology Letters*, 2: 470–474.
- Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA11: Molecular evolutionary genetic analysis version 11. *Molecular Biology and Evolution*, 38: 3022–3027.
- Zainudin, R., MD Zain, B.M., Ahmad, A. & M.Nor, S. 2017. Microhabitat partitioning of closely related Sarawak (Malaysian Borneo) frog species previously assigned to the genus *Hylarana* (Amphibia: Anura). *Turkish Journal of Zoology*, 41: 876–891.

APPENDIX

Comparative material examined:

C. chalconota: Java [MZB Amph 32568-32570, 32580 from West Java, Bogor Regency, Megamendung District, Cipayung Datar, Pasir Angin, Wikasatrian; MZB Amph 27330, 27350 from West Java, Sukabumi Regency, Sukamanis Village; MZB Amph 4656, 5344 from West Java, Mountain Halimun, MZB Amph 29927, 29937 from West Java, Gede Pangrango Mountain; MZB Amph 15358-15259, from Central Java, Batu Raden, Cilacap; MZB Amph 15970, 15976 from Central Java, Slamet Mountain; MZB Amph 16376, 16380, 16385 from Central Java, Unggaran Banyuwindu; MZB Amph 23209-23210 from Central Java, Banyumas Regency, Singasari Village; MZB Amph 92, 21357 from Bali].

C. megalonesa: Java [MZB Amph 32573-325677 from West Java, Bogor Regency, Megamendung District, Cipayung Datar, Pasir Angin, Wikasatrian; MZB Amph 23111-23112 from West Java, Cibinong, LIPI Area; MZB Amph 5353,5357 from West Java, Halimun mountain, Sungai Cikaniki; MZB Amph 21393,21395-21397,21399 from West Java, Sukabumi Regency, Cimanggu Village; MZB Amph 11002, 11004 from West Java, Cigadog Village, Halimun Mountain National Park; MZB Amph 5353, from West Java, Halimun Mountain, Sungai Sukawayana; MZB Amph 27311West Java, Regency Sukabumi, Sukamanis Village; MZB 29907 Amph West Java, Gede Pangrango Mountain; MZB Amph 15356 Central Java, Batu Raden, Cilacap; MZB Amph 16677 East Java, TAHURA Raden Soerjo, National Park].

C. megalonesa: Borneo [MZB Amph 3566, 21521 from West Kalimantan, Bentuang Karimun, Menjakan Putusibou; MZB Amph 31973 from West Kalimantan, Dawar Regency, Marom Village; MZB Amph 7991, 7994, 7995, 7999 from East Kalimantan, Bulungan; MZB Amph 21522-21523 from Central Kalimantan, Sungai Kalang; MZB Amph 9997-9998 from East Kalimantan, Karimun, Sungai Ulumantang Bentuang; MZB Amph 6212, 6215 from East Kalimantan, Muruai; MZB Amph 3969, 3995 from Kalimantan, Karimun, Sungai Takalan; MZB Amph 10012, 10014 from Kalimantan, Karimun, Bentuang; MZB Amph 25470, 25496, 25498 from Kalimantan, Meratus].