# Systematics of Eutropis rugifera (Stoliczka, 1870) (Squamata: Scincidae) including the redescription of the holotype 

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#### Abstract

Eutropis rugifera has long been identified as a widespread species complex distributed in Nicobar, Peninsular Malaysia, Greater Sundaic Islands, Bali, Sulawesi and the Philippines. This skink was described by Stoliczka in 1870 from Nicobar Island based on a single specimen (holotype by monotypy). Later, Peters (1871), Bartlett (1895) and Werner (1896) described three more species which were morphologically similar to Euprepes percarinatus (from Java), Mabuia rubricollis (Borneo) and M. quinquecarinata (Sumatra) respectively, which are currently considered junior objective synonyms of Eutropis rugifera. We examined all the available synonym types and voucher specimens of Eutropis rugifera deposited at several museums. A morphological examination of the types of this species and mtDNA analysis ( 584 bp of 16S rRNA) of the samples from different biogeographic regions revealed that Eutropis rugifera from Nicobar Island, Bali Island, and Bawean Island are composed of a monophyletic species. However, the taxonomic status of the above population requires further clarification, and the population in Bawean Island may represent a cryptic species. Finally, we provide a complete redescription of E. rugifera based on its holotype.


Key words: biogeography, cryptic species, rough-scaled sun skink, species complex, Sundaland

## Introduction

The quinque-carinate (rarely seven keeled) skink, Tiliqua rugifera was described by Stoliczka (1870) based on a single specimen (Zoological Survey of India, Kolkata, ZSI 2350) collected from Camorta, Nicobar Island. Peters (1871) placed T. rugifera in the genus Euprepes, and described a closely related species, Euprepes percarinatus from Malang, East Java based on two specimens (Museum für Naturkunde, Berlin, ZMB 5442, 5442A). Furthermore, he introduced a variation, Euprepes percarinatus var. borneensis based on two specimens collected from Sarawak, Borneo. Later, Boulenger (1887) synonymized the latter species including its variation with $E$. rugifera, and transferred the forma typica into the genus Mabuia. Bartlett (1895) described the Bornean variation as Mabuia rubricollis based on three specimens (Natural History Museum, London, BMNH 1946.8.18.22-24) collected from Kuching, Borneo. Werner (1896) described another closely related species, M. quinquecarinata from Sumatra based on a single specimen (ZMB 12031). However, both species were synonymized with M. rugifera by Smith (1935). Later, Horton (1973) transferred this species into a new genus, Dasamia. Greer (1977) restored the former generic status. Subsequently, Asian Mabuya species were placed in the genus Eutropis by Mausfeld et al. (2000). The following authors recorded Eutropis rugifera from various locations in Nicobar Island,

Thailand, Peninsular Malaysia, and Greater Sundaic Islands (Sumatra, Borneo, Java, and Bali), Sulawesi and surrounding islands of the above locations (Mertens 1959; Cox et al. 1998; Chan-ard et al. 1999; Malkmus et al. 2002; Das 2004; Onn et al. 2010; Venugopal 2010; Grismer 2011; Barley et al. 2014 etc.). Eutropis rugifera has long been considered a widely distributed species and the taxonomy of the species has not yet been critically compared. Therefore, we examined the morphology of E. rugifera specimens at various museum collections, and hereby provide preliminary data on the mtDNA variation of this complex across the different biogeographic regions. Furthermore, we provide a redescription of Eutropis rugifera based on the holotype as its original description is short and lacking most of the important diagnostic characters.

## Materials and methods

We compared specimens and the description of all known congeners of this species complex including its synonym types (Appendix). We examined specimens from the following collections: Natural History Museum, London, UK (BMNH); Naturmuseum Senckenberg, Frankfurt, Germany (SMF); Museum für Naturkunde, Berlin, Germany (ZMB); Zoological Survey of India, Kolkata, India (ZSI); Museum of Zoology, Research Center for Climate Change, University of Indonesia (UIMZ); and Zoologisches Museum Hamburg, Germany (ZMH). Museum acronyms follow Sabaj (2016). We obtained distribution data from examined specimens as well as published literature. With a Mitutoyo digital caliper ( $\pm 0.1 \mathrm{~mm}$ ) and a Leica Wild M3Z dissecting microscope, we measured the following characters on the left side of the body: snout-vent length (SVL, from tip of snout to anterior margin of vent), thigh length (FEL, from the anterior margin of the hind limb at its insertion point on the body to the knee while flexed), shank length (TBL, from the posterior surface of the knee while flexed to the base of the heel), head length (HL, from posterior edge of mandible to tip of snout); head width (HW, maximum width of head at the angle of the jaws), orbit diameter (ED, the greatest horizontal diameter of the orbit); tympanum-eye length (TYE, from posterior border of orbit to anterior border of tympanum), snout length (ES, from anterior border of orbit to tip of snout), eye-nostril length (EN, from anterior border of orbit to middle of nostril), toe lengths (TL, from tip of claw to the nearest fork). We counted supralabial and infralabial scales from the gape of the lips to the rostral and mental scales respectively. Our counts of ventrals include all scales from the postmental to the last scale bordering the vent. We counted paravertebral scales between postparietal (included) to the posterior margin of the thigh in a straight line immediately left of the vertebral column. We counted subdigital lamellae on Toe IV from the first proximal enlarged scansor wider than the width of the largest palm scale to the distal-most lamella at the base of the claw. We counted the number of longitudinal scale rows (ventral and dorsal) at midbody. We sexed specimens by the presence of everted hemipenes or by ventral tail dissection.

Total genomic DNA was extracted from liver tissues using the standard phenol-chloroform method (see Sambrook et al. 1989) and the Diatom DNA Prep 100 Tissue Kit (IsoGene) following the manufacturer instructions. The obtained DNA was purified, visualized in UV-light electrophoresis and the resultant concentration was measured. The final concentration of DNA was adjusted to approximately $100 \mathrm{ng} / \mu \mathrm{L}$ using NanoDrop 2000 (Thermo Scientific). We amplified the 452 bp fragment of the 16 S rRNA, a mitochondrial gene proved to be most useful for species identification in reptiles; PCRs and sequencing were performed after the protocol of Poyarkov et al. (2015a, b). We used the primers 16 sar-L (forward; $5^{\prime}$-CGC CTG TTT ATC AAA AAC AT-3') and 16 sbr-H (reverse; $5^{\prime}$-CCG GTC TGA ACT CAG ATC ACG T-3') of Palumbi et al. (1991) to amplify a section of the mitochondrial 16 S ribosomal RNA gene. PCR was conducted using the GenePack Core (IsoGene) in a total volume of $20 \mu \mathrm{l}$, which contained from 1.0 to $5.0 \mu \mathrm{l}$ of DNA extraction, $1.0 \mu \mathrm{l}$ of forward and reverse primers $(10 \mu \mathrm{M}), 0.2 \mu \mathrm{l}$ of Taq polymerase $(5 \mathrm{U} / \mathrm{ml}), 1 \mu \mathrm{dNTP}$ mix, and PCR buffer containing 15 mM MgCl 2 . PCR conditions were as follows: initial denaturation for 5 min at $94^{\circ} \mathrm{C}, 31-35$ cycles composed of the 30 seconds denaturation at $94^{\circ} \mathrm{C}, 45$ seconds of annealing at $50-57^{\circ} \mathrm{C}$, and 1 min extension at $72^{\circ} \mathrm{C}$. PCR products were purified with the Wizard SV Gel and PCR Clean-up System (Promega).

The forward and reverse sequences were checked visually in Chromas 2.0 and a consensus sequence was compiled with BioEdit 5.0.9 (Hall 1999). Nucleotide sequences were initially aligned using ClustalX 1.81 (Thompson et al. 1997) with default parameters and optimized manually in BioEdit 7.0.5.2 (Hall 1999) and MEGA 5.0 (Tamura et al. 2011). Mean uncorrected genetic distances ( $p$-distances) between sequences were determined with MEGA 5.0 (Tamura et al. 2011). MODELTEST version3.06 (Posada \& Crandall 1998) was used to estimate
the optimal evolutionary model to be used for the data set analysis. The preferred model was the GTR $+\Gamma+\mathrm{I}$ model, as suggested by the Akaike information criterion (AIC). Phylogenetic trees were inferred using two alternative methods, Maximum Likelihood (ML) and Bayesian analyses (BI). The ML analyses were conducted using Treefinder (Jobb et al. 2004). Transitions and transversions were equally weighted, and gaps were treated as missing data. The BI analyses were carried out with MrBayes version 3.1.2 (Huelsenbeck \& Ronquist 2001; Ronquist \& Huelsenbeck 2003). The analysis was run with four chains to proceed for twenty million generations and trees were sampled for every 1000 generations. Analyses were repeated beginning with varying starting trees to ensure that our analysis was not restricted from the global optimum. To confirm that the chains had achieved stationarity, we evaluated "burn-in" plots by plotting log-likelihood scores and tree lengths against the generation number using the software Tracer v.1.5 (Drummond \& Rambaut 2007). The first eight million generations, before this chain apparently reaching stationarity, were discarded. A majority rule consensus tree (BI tree) was calculated from the posterior distribution of trees. Confidence in tree topology was tested by non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replicates for the ML, and by posterior probability (PP) for BI in MrBayes 3.1.2 (Huelsenbeck \& Ronquist 2001). Branches with bootstrap values $70 \%$ or greater and BI PP values over 0.95 were regarded as being strongly supported (Huelsenbeck \& Hillis 1993).

Sequences were submitted to a BLAST search in GenBank to confirm the intended sequences had been amplified. The obtained sequences are deposited in GenBank under the accession numbers KY515234-KY515239 (see Table 2).

## Results

Statistically informative morphometric tests could not be performed because of the small sample size of each population. Nonetheless, the diagnostic morphometric and meristic data taken for the type specimens and the interspecific comparisons are summarized in Table 1. We redescribe E. rugifera Stoliczka (1870) here based on the holotype (ZSI 2350) (see Systematics).

For a preliminary assessment of the geographic variation of mtDNA markers within the Eutropis rugifera complex, we analyzed sequences of 10 specimens of $E$. rugifera from India (Nicobar Island, type locality of $E$. rugifera) and Indonesia, including Bali and the Bawean Islands (see Tables 2, 3, Fig. 1). The outgroup included 16 S rRNA sequences of 18 congeneric taxa of Eutropis from South and Southeast Asia, two more distant lygosomine skinks of the genera Lipinia, Mabuya, and Trachylepis (for references and voucher details see Table 2).

The final alignment used for the phylogenetic analysis comprised $584 \mathrm{bp}, 389$ of the sites were found to be conserved, 183 variable and 126 parsimony-informative; the transition-transversion bias was estimated at 1.481 (all data given for ingroup only).

The results of the phylogenetic analysis of the 16 S rRNA gene are shown in Figure 1. Phylogenetic relationships between the accessed taxa of Eutropis are poorly resolved with three major basal nodes in the tree having low ( $\mathrm{BS}<75 \%$ ) or negligible levels ( $\mathrm{BS}<50 \%$; BPP $<0.95$ ) of support, whereas monophyly of the genus Eutropis and monophyly of species-level groups and species complexes are significantly supported ( $\mathrm{BS}>90 \%$; BPP $\geq 0.95$ ). Both the ML and Bayesian analyses resulted in essentially similar topologies (see Fig. 1).

The general topology of the phylogenetic relationships resulting from our analyses is consistent with results reported in previous studies (Mausfeld \& Schmitz 2003; Datta-Roy et al. 2012; Barley et al. 2014). Maximum likelihood analyses (Fig. 1) infer the following set of phylogenetic relationships among studied Eutropis species:
(1) Monophyly of the genus Eutropis is well supported by all types of analyses ( $95 / 1.0$; hereafter node support values are given for BS/PP respectively).
(2) E. multifasciata and E. longicaudata are clustered together and form a sister clade to all other species of Eutropis included in our analysis; monophyly of this clade is not significantly supported by Bayesian Analysis (PP $=0.85)$ but is significantly supported in ML analysis $(\mathrm{BS}=95)$.
(3) The E. rugifera complex members form a well-supported monophyly ( $\mathrm{BS} / \mathrm{PP}=100 / 1.0$ ), well distinct from all other Eutropis species, and is therefore recovered as a sister clade with respect to all other sampled Eutropis, except for $E$. multifasciata and E. longicaudata (Fig. 1).
(4) The phylogenetic position of E. tytleri is not recovered; the remaining species of Eutropis from India and Southeast Asia form a poorly supported monophyly (65/0.95).
(5) Monophyly of the Philippine group consisting of E. indeprensa + E. cumingi + E. multicarinata, as well as of the clade joining $E$. rudis and $E$. macrophtalma from Sundaland is well supported ( $\mathrm{BS}>75 \% ; \mathrm{PP}>0.95$ ).
(6) Indian taxa E. carinata + E. beddomei + E. nagarjuni + E. trivittata from a significantly supported clade (86/0.99).
(7) The Indian species, E. macularia is phylogenetically associated with other Indian species such as $E$. clivicola and E. allapallensis $(43 / 0.95)$ and is clearly evolutionarily distant from E. rugifera complex.

Our analyses recover three highly-supported clades within the E. rugifera complex; however, relationships among the three clades remain poorly supported (Fig. 1). The first clade joins the sample from Nicobar Island (India), the type locality of E. rugifera, with two samples from Indonesia (Sumatra); it corresponds to the type $E$. rugifera s. str. (Fig. 1, clade 1). The other two clades correspond to Indonesian island populations from Bawean (Fig. 1, clade 2) and Bali (Fig. 1, clade 3) and are reciprocally monophyletic.

The uncorrected p-distances among and within the 16 S rRNA gene fragment of the studied Eutropis species are shown in the Table 3. The observed $p$-distances among members of $E$. rugifera species complex varied from $2.8 \%$ to $4.3 \%$ of substitutions.


FIGURE 1. The ML tree resulting from analysis of the studied samples of Eutropis. For the major cladogenetic events ML BS/ BI PP values are given.

## Systematics

## Eutropis rugifera (Stoliczka, 1870)

(Figs. 1-4; Tables 1-3)

Tiliqua rugifera Stoliczka, 1870 (type locality: Camorta, Nicobar)
Euprepes percarinatus Peters, 1871 (type locality: Malang, East Java) Mabuia rubricollis Bartlett, 1895 (type locality: Kuching, Borneo) Mabuia quinquecarinata Werner, 1896 (type locality: Sumatra)


FIGURE 2. Photographs in life of adult Eutropis rugifera (UIMZ 0051, SVL 56.7 mm ) from the Bali Barat National Park, Bali, Indonesia showing (A) dorsal, (B) ventral, and (C) lateral views of the head (Photo: RCCC-UI).

Holotype. Adult male (ZSI 2350), collected from Camorta, Nicobar Island ( 8.1299 N, 93.4902 E) by Ferdinand Stoliczka, in 1870 (exact date not given). The type specimen was registered in the Indian Museums on 24 July 1878.

Redescription of holotype. Male, SVL 52.9 mm . Head moderately large (HL $24.9 \%$ SVL, HL $50.2 \%$ of axilla-groin distance), narrow (HW 56.1\% HL, HW $14.0 \%$ SVL), indistinct from neck; snout short (snout length $34.1 \%$ of head length, snout length $60.8 \%$ of head width), slightly convex in lateral profile; rostral shield large, hemispherical, distinctly visible from above, posterior margin of midpoint curved; frontonasal broadly in contact with rostral; frontonasal wide, lateral border touching first loreal; prefrontals separated from each other, largest distance along the longitudinal axis of frontonasal equal to prefrontals in length, lower border touching both loreal scales, the posterior border touching the first supraocular, and frontal; frontal large, elongate, subtriangular, bluntly pointed posteriorly, equal in length to the frontoparietals and interparietal combined; frontoparietals two, in contact, distinct, larger than interparietal; parietals damaged (examined materials have parietals strongly keeled, large, and broadly in contact and overlapping behind the interparietal, touching pretemporal scales laterally); nuchals damaged (examined materials have a single pair of nuchals, overlapping middorsally behind interparietal). Nostril large and situated in the middle of the nasal; single supranasal; two loreals, anterior loreal touching nasal, supranasal, frontonasal, prefrontal, posterior loreal, and $2^{\text {nd }}$ supralabial; posterior loreal longer than the anterior loreal in the antero-posterior axis, touching prefrontal and first supracilliary; two presuboculars; eye large (orbit diameter $27.3 \%$ head length), orbit diameter equal in length to tympanum-eye length, pupil rounded; interorbital distance broad; three small postoculars; four wide supraoculars, the second is the longest in the antero-posterior axis and the widest in the sagital axis and fully contacts the frontal; first supraocular in contact with prefrontal and frontal, $2^{\text {nd }}$ in contact with frontal and frontoparietal, $3^{\text {rd }}$ in contact with frontoparietals, and $4^{\text {th }}$ in contact with both frontoparietal and parietal; supracilliaries six; eyelid moveable with a scaly window composed of seven scales. Six supralabials, fifth largest and at the mid orbit position ( $5^{\text {th }}$ touching eye); three pretemporals; two primary temporals, three secondary temporals; infralabials six; ear opening deep, small, near spherical and approximately one quarter of eye diameter. Mental large; a single large postmental followed by two chin shield pairs, the first pair meeting in midline, the first chinshield in contact with first and second infralabial scales, the second pair in contact with second and third infralabials.


FIGURE 3. Photograph of the habitat of Eutropis rugifera in the Bali Barat National Park, Bali, Indonesia (Photo: A. A. Thasun Amarasinghe).

TABLE 1. Morphometric (in mm ) and meristric character comparison of the Eutropis rugifera (Stoliczka, 1870) holotype, its junior synonym types, and other materials; "-" not measured.

| Locality | Nicobar | Java | Borneo | Sumatra | Bali | Bawean Is. |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Examined material | Tiliqua <br> rugifera | Euprepes <br> percarinatus <br> Syntypes <br> $(n=2)$ | Mabuia <br> rubricollis <br> Syntypes <br> $(n=3)$ | Mabuia <br> quinquecarinata <br> Holotype | Eutropis <br> rugifera <br> $(n=8)$ | Eutropis <br> rugifera <br> $(n=7)$ |
|  |  | 52.9 | $50.7-53.0$ | $54.5-57.6$ | 57.0 | $46.1-56.7$ |
| SVL | 13.2 | $11.8-13.3$ | $13.0-13.7$ | 11.2 | $12.5-14.8$ | $12.3-15.9$ |
| Head length | 7.4 | $8.0-8.3$ | $9.5-11.4$ | 8.9 | $7.6-9.0$ | $7.3-9.7$ |
| Head width | 4.5 | $4.3-4.5$ | $4.8-5.0$ | 4.3 | $4.0-4.8$ | $4.0-5.2$ |
| Snout length | 3.6 | $2.5-2.6$ | $3.1-3.9$ | 2.9 | $3.0-4.2$ | $3.7-4.2$ |
| Orbit diameter | 3.7 | $4.6-4.7$ | $4.0-4.9$ | 4.6 | $2.7-3.7$ | $2.3-3.7$ |
| Eye-tympanum distance | 26.3 | $23.1-23.6$ | $21.7-27.3$ | 20.6 | - | - |
| Axilla-groin distance | 8.1 | $7.8-8.4$ | $8.2-11.5$ | 10.2 | $6.0-8.4$ | $6.8-9.5$ |
| Thigh length | 7.5 | $7.8-8.4$ | $6.8-9.5$ | 9.5 | $6.7-8.4$ | $7.0-8.8$ |
| Shank length | 5 | 5 | 5 | 5 | $5-7$ |  |
| Number of dorsal keels | 5 | 26 | $26-28$ | 28 | $26-28$ | $28-30$ |
| Midbody scale rows | $25+$ | $30+$ | $30-32$ | damaged | 30 | $30-33$ |
| Paravertebral scales | $30+$ | $43-43$ | 21,22 | 23,24 | 22 | $30-33$ |
| Ventrals | 43 | 1 | 1 | 1 | $22-45$ | $42-45$ |
| Lamellae on Toe IV | 25 | 0 | 0 | 1 | $21-25$ |  |
| Nuchal present $(1)$, absent $(0)$ | 1 |  |  | 0 | 1,0 |  |
| Prefrontals separated $(0)$, in | 0 |  |  |  | 0,1 |  |
| contact (1) |  |  |  |  |  |  |



FIGURE 4. Current distribution of typical Eutropis rugifera (grey shading); type locality of E. rugifera (red), and synonyms of E. rugifera (blue): 1. Euprepes percarinatus, 2. Mabuia rubricollis, 3. Mabuia quinquecarinata; other sampling localities of this study show in green: Bawean and Bali Islands.


FIGURE 5. Photographs in life of adult Eutropis rugifera showing intraspecific variation of dorsal colouration among individuals from the Bali Barat National Park, Bali, Indonesia (A) UIMZ 0051 (SVL 56.7 mm ) with vivid colour pattern, (B) UIMZ 0062 (SVL 51.8 mm ) with uniform colour pattern, and (C) UIMZ 0064 (SVL 49.6 mm ) with typical colour pattern (Photo: RCCC-UI).

Body scales have five keels with three median prominent and two secondary keels per scale; all scales imbricate and lack apical pits; scales on the dorsal surface of thigh keeled; body slender, elongate (axilla-groin distance $49.7 \% \mathrm{SVL}$ ); midbody scale rows $25+$ (damaged); paravertebral scales $30+$ (damaged); six enlarged preanal scales.
TABLE 2. Voucher and GenBank information on the specimens used in this study. GenBank accession numbers, locality information and countries of origin are given.

| Species | GenBank AN | Voucher ID | Locality | Reference |
| :---: | :---: | :---: | :---: | :---: |
| Eutropis allapallensis | JQ767961 | CES09/851 | India, Orissa, Satkosia WLS | Datta-Roy et al., 2012 |
| Eutropis beddomii | JQ767965 | CES09/943 | India, Orissa, Deomali | Datta-Roy et al., 2012 |
| Eutropis bibronii | JQ767963 | CES09/949 | Rushikulya, Orissa, India | Datta-Roy et al., 2012 |
| Eutropis carinata | JQ767953 | CES08/809 | India, Karnataka, B.R.T WLS | Datta-Roy et al., 2012 |
| Eutropis clivicola | JQ767956 | CESL089 | India, Kerala, Parambikulam WLS | Datta-Roy et al., 2012 |
| Eutropis cumingi | DQ238896 | FMNH 259457 | Philippines, Luzon | Whiting et al., 2006 |
| Eutropis indeprensa | AY159076 | ZFMK73807 | Philippines | Mausfeld \& Schmitz, 2003 |
| Eutropis longicaudata | DQ238898 | FMNH255526 | - | Whiting et al., 2006 |
| Eutropis longicaudata | AY070359 | ZFMK 71273 | Vietnam, Phong Nha - Ke Bang | Mausfeld et al., 2002 |
| Eutropis macrophtalma | AY159077 | ZFMK71716 | Indonesia | Mausfeld \& Schmitz, 2003 |
| Eutropis macularia | AY159078 | CAS212475 | Myanmar, Mwe Hauk village | Mausfeld \& Schmitz, 2003 |
| Eutropis madaraszi | AY159080 | ZFMK15976 | Sri Lanka, Gammaduwa | Mausfeld \& Schmitz, 2003 |
| Eutropis multicarinata | AY159081 | USNM54660 | Philippines | Mausfeld \& Schmitz, 2003 |
| Eutropis multifasciata | DQ238897 | FMNH 255530 | - | Whiting et al., 2006 |
| Eutropis nagarjuni | JQ767952 | NHM.OU.REP.3-2009 | India, Andhra Pradesh, Nagarjunasagar-Srisailam TR | Datta-Roy et al., 2012 |
| Eutropis quadricarinata | AY159089 | USNM520616 | Myanmar, Chattin WLS | Mausfeld \& Schmitz, 2003 |
| Eutropis rudis | AB028790 | - | Indonesia, Sulawesi | Honda et al., 2003 |
| Eutropis rugifera | AY159090 | - | Indonesia, no exact locality | Mausfeld \& Schmitz, 2003 |
| Eutropis rugifera | AY159079 | ZFMK66754 | Indonesia, Sumatra | Mausfeld \& Schmitz, 2003 |
| Eutropis rugifera | JQ767962 | CES09/897 | India, Nicobar Island | Datta-Roy et al., 2012 |
| Eutropis rugifera | AY159093 | ZFMK76383 | Indonesia, Bali Island | Mausfeld \& Schmitz, 2003 |
| Eutropis rugifera | KY515234 | UIMZ 0062 | Indonesia, Bali Island | this paper |
| Eutropis rugifera | KY515235 | UIMZ 0064 | Indonesia, Bali Island | this paper |
| Eutropis rugifera | KY515236 | UIMZ 0070 | Indonesia, Bali Island | this paper |
| Eutropis rugifera | KY515237 | UIMZ 0106 | Indonesia, Bawean Island | this paper |
| Eutropis rugifera | KY515238 | UIMZ 0107 | Indonesia, Bawean Island | this paper |
| Eutropis trivittata | JQ767951 | CES09/976 | India, Maharashtra, Satara | Datta-Roy et al., 2012 |
| Eutropis tytleri | AY159074 | ZFMK73431 | India | Mausfeld \& Schmitz, 2003 |
| Lipinia vittigera | KY515239 | ZMMU R-14477 | Thailand, Surat Thani | this paper |
| Mabuya agilis | AY070347 | - | Brazil, Bahia, Prado | Mausfeld et al., 2002 |
| Trachylepis quinquetaeniata | DQ238942 | BYU 47330 | Ghana, USFWS | Whiting et al., 2006 |

TABLE 3．Mean uncorrected genetic p－distances distances（percentage）between（below diagonal）the different lineages revealed within Eutropis rugifera complex and an outgroup taxa．The standard error （ 500 bootstrap replicates）is given above diagonal．Clade numbers correspond to those at Figure 1 and Table

|  | で8 | て＇II | $\varsigma^{\prime}$ II | でてI | $\dagger \mathrm{I}$ | $S^{\prime}$ II | 66 | で0I | ZI | $\varsigma^{\prime}$ II | 8＇ZI | ¢＇0I | で0I | L＇I I | ZI | $\varsigma^{\prime}$ II | で0I | t＊ 6 | て＇01 | ZI | L＇0I | で0I | pḰnqpW | Ez |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\pm 1$ |  | で0I | て＇II | で0I | $\varepsilon 1$ | でII | L＇8 | L．6 | $\varsigma^{\prime}$ II | 66 | $\varepsilon[$ | L＇II | で6 | L．6 | 6.6 | ¢ 01 | カ88 | L＇8 | †6 | 6.6 | ャ6 | で8 | s！dәјイцวр．и | 27 |
|  |  |  | $8 \cdot \downarrow$ | $8{ }^{\circ}$ | 66 | $6{ }^{\circ} \mathrm{L}$ | で8 | †6 | t＊ | で6 | でII | で6 | $L \cdot L$ | †6 | L＇8 | L＇8 | $6{ }^{\circ}$ | $L \cdot L$ | L＇8 | L＇6 | ع＇8 | ガL | ！8u！ | IZ |
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| £Z | 72 | IZ | 07 | 6I | 8I | LI | 9 I | SI | tI | EI | ZI | II | 0I | 6 | 8 | $L$ | 9 | ¢ | $t$ | $\mathcal{E}$ | $\checkmark$ | I | səりədS |  |



FIGURE 6. Photographs in life of adult Eutropis rugifera showing intraspecific variation of dorsal head scale patterns among individuals from Bawean Island, Indonesia (A) UIMZ 0106, (B) UIMZ 0107 (Photo: UIMZ).

Forelimbs short; hind limbs relatively long (FEL 15.3\% SVL, TBL 14.2\% SVL); shank slightly shorter, 92.6\% FEL; dorsal surfaces of fore and hind limbs keeled; subdigital lamellae on Toe IV 25; relative length of fingers: IV $>$ III $>$ II $>\mathrm{V}>$ I; relative length of toes: IV $>$ III $>\mathrm{V}>$ II $>$ I. Tail complete, distal part regenerated; median subcaudal scale row of original tail subequal, median subcaudal scale row of regenerated tail enlarged, wider than long.

Colouration in preservative. Colouration and markings almost faded; dorsal head, body and limbs uniform brown; Venter uniform cream coloured.

Colouration in life. (Fig. 2) for live colouration of Eutropis rugifera based on UIMZ 0051 in life in the Bali Barat National Park, Bali, Indonesia. Dorsal head, body and limbs chestnut brown; dorsal and ventral head with several black spots; a dark vertebral stripe on the back separated by a median pale stripe, commencing from posterior occipital area; two additional dark stripes situated laterally, commencing from snout; the vertebral stripe descends down to posterior end of body then fades away; the lateral stripes extend to the hind limbs. Lateral body creamy brown, and belly creamy white while ventral head sky blue.

Habitat and Natural History. A fast-moving, litter dwelling skink, usually observed in dry mixed habitats with a thin layer of leaf litter on the forest floor, with semi-open canopy (canopy cover $50 \%$; Fig. 3), and mostly observed in the morning (06:00-08:00 hr) and evening (16:00-18:00 hr). Almost all the colour variations (with multi-stripes, two stripes, without stripes etc.) found sympatrically, usually found in the same pitfall trap network ( $25 \mathrm{~m}^{2}$ area). Larger specimens tried to bite during handling and moved rapidly to escape. While handling their body scales loosened (and some scales fell off the body), this may be an escape strategy used by this species to escape from the predator. Insects should be the main food; because ants, dipterans, and orthopterans were the usual gut contents.

## Discussion

Although several species were elevated from Borneo, Sumatra, and Java, Eutropis rugifera has long been considered a single species distributed from Nicobar, Malaysia Peninsula, and the Greater Sundaic Islands (Fig. 4).

However, Smith (1935) previously observed some morphological differences between populations (e.g. a specimen from Mt. Kledang, Perak, Peninsular Malaysia at $\sim 800 \mathrm{~m}$ elevation). Moreover, Horton (1973) considered this species as belonging to a separate genus, Dasamia, and transferred it to this new genus along with Tiliqua multicarinata (Gray, 1845). Greer (1977) restored the former generic arrangement and Horton's generic allocation was rejected by all subsequent researchers except Das (1996). Although E. rugifera and E. multicarinata are morphologically similar to each other (Table 2) the morphological characters are not strong enough to remove them (E. rugifera and E. multicarinata) from the genus Eutropis. Both species share the same typical morphological characters of the genus Eutropis. Mausfeld and Schmitz (2003) clearly stated (based on their molecular results) that E. rugifera belongs to the genus Eutropis, and that it has no close affinity at all to E. multicarinata. Therefore, the genus Dasamia remains a synonym of the genus Eutropis. Horton (1973) states that E. rugifera has 24-30 midbody scale rows and $5-11$ keels on its dorsal scales, which obviously, suggests a species complex. It is very unusual to see meristic characters expressed in such a broad range (Table 1), this strongly suggests that there may be some irregularities in Horton's (1973) identifications.

The mitochondrial gene, 16S rRNA, proved to be useful for species identification in reptiles; this gene has been proven to be informative in resolving the relationships within squamates (Reeder \& Wiens 1996; Pellegrino et al. 2001; Whiting et al. 2003, 2006), including Lygosomine skinks and the genus Eutropis (Mausfeld et al. 2002; Honda et al. 2003; Mausfeld \& Schmitz 2003; Whiting et al. 2006; Datta-Roy et al. 2012). Our data confirm previously published results on the phylogenetic position of the $E$. rugifera species complex as one of the basal lineages within the differentiation of Southeast Asian Eutropis; despite superficial morphological similarity, E. rugifera appears to be phylogenetically distant to $E$. macularia from the Indian subcontinent and $E$. quadricarinata from Myanmar (Mausfeld \& Schmitz 2003; Datta-Roy et al. 2012; Barley et al. 2014). Previous studies also reported certain differentiation within the E. rugifera complex. Mausfeld \& Schmitz (2003) noted the substantial genetic difference between the Balinese population and other Indonesian E. rugifera specimens, and further suggested that the genetic difference between the Balinese population and the other E. rugifera specimens may indicate that the populations on Bali have been isolated for a substantially long time (although the sea passage between East Java and Bali is narrow and should not hinder species migration). Apart from the genetic difference, Mausfeld and Schmitz (2003) stated that Balinese specimens have conspicuous and unusual colouration in comparison to the 'typical' E. rugifera form. Despite conspicuous colouration (Balinese population E. rugifera are uniform brown dorsally and laterally, lacking the typical 5-7 greenish-white longitudinal lines or white lines broken up into series of spots, see Smith, 1935), Mausfeld \& Schmitz (2003) assigned the Balinese population to $E$. rugifera. However, we have observed several individuals with two uniform brown, and interrupted longitudinal lines, also with 5-7 longitudinal lines within one population (Fig. 5). Furthermore, the characters of pholidosis agree with the holotype from Nicobar (see Table 2).

Recently, Datta-Roy et al. (2012) included in their analysis a sample of E. rugifera from Nicobar Island, the type locality, and they also reported certain divergence between sequences of this specimen and a sample from Bali, Indonesia. More recently, Barley et al. (2014) analysed the biogeography of the genus Eutropis and reported significant divergence between the E. rugifera samples from the Philippines (Mindanao Island, a newly discovered population) and a clade joining samples from Sulawesi Island (Indonesia), Sarawak, Borneo Island (Malaysia) and Johor, peninsular Malaysia. The newly discovered Philippine population is, however, highly divergent from the other $E$. rugifera populations (c. 17-18\% uncorrected $p$-distance for ND2) and most probably represents a distinct yet undescribed species (Barley et al. 2014).

Unfortunately, we have not managed to compare the mtDNA fragment with the data of Barley et al. (2014); however, our results indicate that taxonomic diversity within the $E$. rugifera complex is highly underestimated. Our data imply that there is no significant divergence in 16S rRNA sequences of $E$. rugifera from Nicobar and from Sumatra, Indonesia, implying that the typical E. rugifera inhabits at least some of the Greater Sunda Islands (Fig. 1, clade 1). We also confirm genetic divergence between the latter clade and the Balinese population of $E$. rugifera ( $p$ distance for 16 S rRNA fragment $2.8 \%$ ), which is slightly less than the interspecific level of genetic divergence for Eutropis (Table 3; Fig. 1, clade 2), thus the taxonomic status of the Balinese E. rugifera will remain a single species.

In this research, for the first time that the previously unstudied population of Eutropis rugifera from Bawean Island, Indonesia, located 150 km into the Java Sea from East Java (Fig. 1, clade 3) has been assessed. Based on our data, this population is characterized with significant genetic divergence in 16S rRNA gene sequences from
both the nominative $E$. rugifera s . str. (Table 3 ; $p$-distance $=3.6 \%$ ) and the Balinese population (Table 3 ; $p$-distance $=4.3 \%$ ). These values are greater than the uncorrected genetic distance values for 16 S rRNA gene observed between several well-recognized species of Eutropis (Table 3), such as E. nagarjuni and E. trivittata ( $p=3.1 \%$ ), E. nagarjuni and $E$. beddomii ( $p=3.1 \%$ ). However, the species identity of these samples (NHM.OU.REP.3-2009, CES09/976, and CES09/943) is uncertain (Amarasinghe et al. 2016a, b). The $4.3 \%$ genetic difference between the Bawean population and the other E. rugifera specimens may indicate that the populations on Bawean have been isolated for a substantial amount of time, bearing significance to the substantially wide sea passages between East Java, Bawean and Kalimantan (Borneo) and paleogeographic evidence (Elmaleh et al., 2004; Metcalf, 2011).

Morphological variation of the Bawean population of E. rugifera was first observed by Mertens (1959). Taking into account the significant morphological differences revealed between rugifera from Bawean and the other $E$. rugifera populations, we assume that the Bawean population has reached the species level of differentiation and could represent a yet undescribed taxon. However, the characters of pholidosis agree with the holotype from Nicobar (Table 2). The morphological characters in the intra-population of Bawean E. rugifera are highly variable (e.g. Fig. 6) thus here we treat the taxonomic status of the Bawean E. rugifera as a single species with its forma typica.

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## APPENDIX I. specimens examined.

Eutropis macrophthalma (Mausfeld and Böhme, 2002): Java (in error), Indonesia: ZFMK 71717 (holotype), ZFMK 71716 (paratype); Sulawesi, Indonesia: MZB 3870-73, 4313, 4314, 4316, 4317, 4319, 4321, 4322 (paratypes of E. grandis), MZB 7785, 1781.
Eutropis multifasciata (Kuhl, 1820): Bali, Indonesia: SMF 22087 (type of Mabuya multifasciata baliensis), MZB 2042, 2100, 8739; Java, Indonesia: MZB 11912 (neotype), 289, 552, 651, 715, 746, 748, 765, 772, 854, 914, 11912-16, 1477, 1495, 1510, 2168, 2170, 2368, 8431, 9419; Karimunjawa, Indonesia: SMF 55147 (type of Mabuya multifasciata tjendikianensis) Sumatra, Indonesia: MZB 1921; Lesser Sunda Islands, Indonesia: MZB 10255; Penang, Malaysia: ZSI 2275-77, 2279, 2280; Thailand: ZSI 18071, 18120, 18121; Myanmar: ZSI 2307, 4633, 4876-79, 11750, 11751, 12630, 12735, 12835, 12836, 16731; Assam, India: ZSI 2285, 2286, 2288, 2306, 2309, 4007, 4625-27, 11416, 11417; Borneo: ZSI 15329, 15336, 15337; Indonesia: BMNH 1946.8.19.54 (type of Tropidolepisma macrurus); India: BMNH 1946.8.19.3 (type of Plestiodon sikkimensis), ZSI 2361-63 (type of Mabuya monticola)
Eutropis rudis (Boulenger, 1887): Sumatra: BMNH 1946.8.15.26 (lectotype); Borneo: BMNH 1946.9.7.46 (paralectotype), ZSI 15330, 15332, 15344, 15345, 20323; Myanmar: ZSI 2307, 12680, 12735, 13474, 24795; Thailand: ZSI 18071.
Eutropis rugifera (Stoliczka, 1870): Camorta, Nicobar Island, India: ZSI 2350 (holotype); Sumatra, Indonesia: ZMB12031 (holotype of Mabuia quinquecarinata); Malang, East Java, Indonesia: ZMB 5442, 5442A (syntypes of Euprepes percarinatus); Java, Indonesia: ZMH R09975; Bali, Indonesia: UIMZ 0051, 0058, 0062, 0064-65, 0068, 0070; Bawean Island, Indonesia: SMF 55181-3, UIMZ 0105-7, 0111; Kuching, Borneo, Malaysia: BMNH 1946.8.18.22-24 (syntypes of Mabuia rubricollis).

