

Genomic and mitochondrial data identify different species boundaries in aposematically polymorphic *Eniclases* (Coleoptera: Lycidae)

Matej Bocek¹, Michal Motyka¹, Dominik Kusy¹, Ladislav Bocak^{1*}

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Supplementary Text.

The brief taxonomic history, morphology, and diversity of *Eniclases* Waterhouse, 1879

The genus *Eniclases* was described by Waterhouse (1879) for a single species *Trichalus luteolus* Waterhouse, 1878 which was a year later described in *Lycus* (gen. 38). Further species were described by Kleine (1926, 1930, 1935). Additional two species were originally described in *Trichalus* by Pic (1921, 1923) and transferred to *Eniclases* by Bocak & Bocakova (1991). The later study additionally added a number of newly described species from New Guinea. The latest study dealing with *Eniclases* added further ten species, mainly from the central part of New Guinea (Bocek & Bocak 2016), and a new species was described from Halmahera (Bocek & Adamkova 2019).

Eniclases shares with other trichaline genera the shortened primary costa 1. Unlike related genera the pronotal carinae form a V-shaped pattern. The male genitalia are characterized by dorsal pigmented line of the phallus and are highly uniform in the whole genus. Therefore, the male genitalia could not be used for identification of species (Bocak & Bocakova, 1991). Most diagnostic characters have been found in the relative size of males eyes and the shape of male antennae. The detailed morphological characteristic of *Eniclases* was provided by Bocak & Bocakova (1991).

At present, *Eniclases* contains 37 species distributed mostly in New Guinea (35 spp.), two species is recorded from the Moluccas. Although *Eniclases* was earlier considered as a characteristic New Guinea lineage and the Moluccan record was supposed to be a result of a recent dispersal event, Bocek & Adamkova (2019) showed that the Moluccan species form a sister to all New Guinean species and that also *Schizotrichalus*, a supposed sister-lineage of *Eniclases*, occurs in the Moluccas. *Eniclases* were intensively collected only in two regions of New Guinea. American entomologists based in the Wau Ecological Institute assembled a large collection from the Owen's and Bismarck Ranges in 1950s and 1960s. The collection was studied in early 1990s (Bocak & Bocakova 1991). Further species were collected in the Central New Guinea in the Baliem valley and northern slopes of the Central Range and these were used for the current study. A few species were described from the Sepik area, Sentani/Jayapura (Hollandia), Manokwari (Dore Bay) and Raja Ampat islands (Misool).

The present analyses do not result in any formal taxonomical changes despite recovered alternative placement of some individuals (Fig. 4). The high intraspecific polymorphism and similarity makes morphology-based identification of *Eniclases* extremely difficult and in some cases neither morphology nor mitochondrial markers provide sufficient information for robust assignment of an individual to a species.

The list of species of *Eniclasses* Waterhouse, 1879.

Eniclasses Waterhouse, 1879: 66

Type species. *Trichalus luteolus* Waterhouse, 1878

Trichalus, subgenus *Trichalolus* Pic, 1923: 36; Bocak & Bocakova, 1991: 206

Type species. *Trichalus (Trichalolus) apertus* Pic, 1923: 36

apertus (Pic, 1923: 36)

Trichalus (Trichalolus) apertus Pic, 1923: 36

=*Eniclasses fumosus* Kleine, 1926: 181; Bocak & Bocakova, 1991: 217

bicolor Bocek & Bocak, 2016: 23

bokondinensis Bocek & Bocak, 2016: 26

brancuccii Bocek & Bocak, 2016: 25

divaricatus (Pic, 1921: 10)

Trichalus divaricatus Pic, 1921: 10

efferatus Kleine, 1926: 181

egregius Kleine, 1926: 181

electus Kleine, 1926: 182

elelimensis Bocek & Bocak, 2016: 26

flabellatus Bocek & Bocakova, 1991: 207

flavoscutellaris Bocek & Bocakova, 1991: 216

fuscicornis Bocek & Bocakova, 1991: 208

infuscatus Bocek & Bocak, 2016: 23

luteolus (Waterhouse, 1878: 113)

Trichalus luteolus Waterhouse, 1878: 113

moluccanus Kleine, 1930: 328

nicricornis Bocek & Bocakova, 1991: 216

niger Bocek & Bocak, 2016: 29

nigriceps Bocek & Bocakova, 1991: 208

nigroruber Kleine, 1935: 318

pallidus Bocek & Bocakova, 1991: 209

papuensis Bocek & Bocakova, 1991: 213

pectinicornis Bocek & Bocakova, 1991: 211

pseudoapertus Bocek & Bocak, 2016: 21

pseudoluteolus Bocek & Bocak, 2016: 29

proximus Bocek & Bocakova, 1991: 209

riedeli Bocek & Bocakova, 1998: 14

robustus Bocek & Bocakova, 1991: 209

sedlaceki Bocek & Bocakova, 1991: 212

serratus Bocek & Bocakova, 1991: 217

similis Bocek & Bocakova, 1991: 210

slipinskii Bocek & Bocakova, 1991: 213

subelectus Bocek & Bocakova, 1991: 215

tikapurensis Bocek & Bocak, 2016: 24

variabilis Bocek & Bocak, 2016: 27

versicolor Kleine, 1926: 182

wauensis Bocek & Bocakova, 1991: 214

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Table S1. The list sampled localities

West Papua Province, Manokwari regency

Mokwam distr.

Maibri vill., Arfak Mts. 1° 05'S 133° 54'E 1570 m

Papua Province

Jayapura regency

Sentani, Cyclop Mts. 02°32.320'S 140°30.738'E 360 m

Sentani, Cyclop Mts. 02°32.487'S 140°30.683'E 275 m

Tolikara regency

Bokondini Distr.

Bokondini 3°40.76'S 138°40.15'E 1287 m

Bokondini 3°40.76'S 138°40.15'E 1250–1300 m

3 km N Bokondini 3°39.741'S 138°40.216'E 1750–1900 m

3 km SW Bokondini 3°42.51'S 138°38.893'E 2100 m

Yalimo regency

Elelim, km 5 rd Apalapsili 3°48.700'S 139°22.088'E 580 m

Elelim, km 6 rd Apalapsili 3°48.686'S 139°21.764'E 650 m

Dombomi, Lower Pass vall. 3°49.477'S 139°10.251'E 1150 m

Central Mamberamo Regency

Kelila distr.

Tikapura (Rd Tagime-Kelila) 3°46.797'S 138°42.933'E 2170 m

Jayawijaya Regency

Wamena distr.

Yiwika, 16 km N Wamena 3°56.883'S 138°57.712'E 2100 m

Table S2. Euclidian distances between *Eniclases* localities in kilometers.
Aggregations designated as AGG1–4, see main
text for further information.

	Sentani 275m	Elelim 580m	Domb 1150m	Bok1 1287m	Bok2 1250m	BokN 2100m	BokSW 1900m	Tikap 2150m	Yiwika 2100m
Sentani	-								
Elelim	189.3	-							
Dombomi	205.8	22.1	-						
Bokondini1	240.3	79.1	58.0	-					
Bokondini2	240.4	79.4	58.3	0.3	-				
Bokondini N	239.3	79.4	58.5	1.9	1.8	-			
Bokondini SW244.0	80.9	59.5	4.0	4.0	5.7	-			
Tikapura	242.1	72.7	50.8	12.3	12.5	14.0	10.7	-	
Yiwika	232.1	47.7	27.0	44.2	44.3	45.2	43.8	33.8	-

Table S3. The list of primers used for mtDNA amplification.

Fragment

Code -mer Sequence (5' >> 3')

rrnL

16a	20	CGCCTGTTAACAAAAACAT
ND1A	27	GGTCCCTTACGAATTGAATATATCCT
ND1-2	24	ATCAAAAGGAGCTCGATTAGTTTC

cox1

JerryN	23	CAACAYYTATTYGATTYTTYGG
MarcyN	24	TTCRTAWGTTCARTATCATTGRTG
JerryM	23	CAACAYYTATTGRTTYTTGG
Marcy	27	TARTTCRTATGWRCAATAYCAYTGRG
SPat	21	GCACTAWTCTGCCATATTAGA
SJerry	23	CAACATYTATTYGATTYTTGG
Pat	25	TCCATTGCACTAATCTGCCATATTA
Jerry	23	CAACATTATTGATTGATT
Marilyn	21	TCATAAGTCAGTATCATTG

nad5

OF1	29	CCTACTCCTGTTCTGCTTAGTCATT
R6	29	GAAACGAAAATCGTATTAAATTGACT
R2M	29	AATTGAASCCAAAAAGAGGTATAC

Table S4. Characteristics of datasets and best-fit models for mtDNA and nextRAD partitions.

mtDNA dataset analysis

Fragment Name	Number of sequences	Sites	Unique	Informative	Constant
<i>rrnL</i>	63	796	99	83	679
<i>cox1</i>	64	1101	227	249	807
<i>nad5</i>	62	1207	272	299	864

Name	Model	LogL	AIC	w-AIC	AICc	w-AICc	BIC	w-BIC
<i>rrnL</i>	TIM3+F+I	-1983.31	4226.64 + 0.0000		4277.86 + 0.0000		4834.9869 + 0.0000	
<i>cox1</i>	TIM2+F+I+G4-4175.6	8617.17 + 0.0000			8654.03 + 0.0000		9282.6984 + 0.0000	
<i>nad5</i>	HKY+F+I+G4-4303.48	8860.96 + 0.0000			8891.09 + 0.0000		9508.1350 + 0.0000	

nextRAD dataset alignment analysis (Wclust = 0.85, MinCov = 4).

Input data: 66 sequences with 6066010 nucleotide sites

Number of constant sites: 5.58659×10^6 (= 92.0966 % of all sites)

Number of invariant (constant or ambiguous constant) sites: 5.58659×10^6 (= 92.0966 % of all sites)

Number of parsimony informative sites: 154300

Number of distinct site patterns: 942024

Model of substitution: GTR+F+I+G4

Rate parameter R:

A-C: 0.9833

A-G: 3.6524

A-T: 0.9157

C-G: 0.9335

C-T: 3.7609

G-T: 1.0000

State frequencies	Rate matrix Q:			
	A	C	G	T
A pi(A) = 0.2936	-0.9256	0.1451	0.6	0.1806
C pi(C) = 0.2047	0.208	-1.103	0.1533	0.7419
G pi(G) = 0.2279	0.7727	0.1377	-1.108	0.1973
T pi(T) = 0.2737	0.1937	0.5548	0.1643	-0.9128

Model of rate heterogeneity: Invar+Gamma with 4 categories

Proportion of invariable sites: 0.6185

Gamma shape alpha: 0.7601

Category	Relative_rate	Proportion
0	0	0.6185
1	0.2282	0.09538
2	1.024	0.09538
3	2.478	0.09538
4	6.755	0.09538

Relative rates are computed as MEAN of the portion of the Gamma distribution falling in the category.

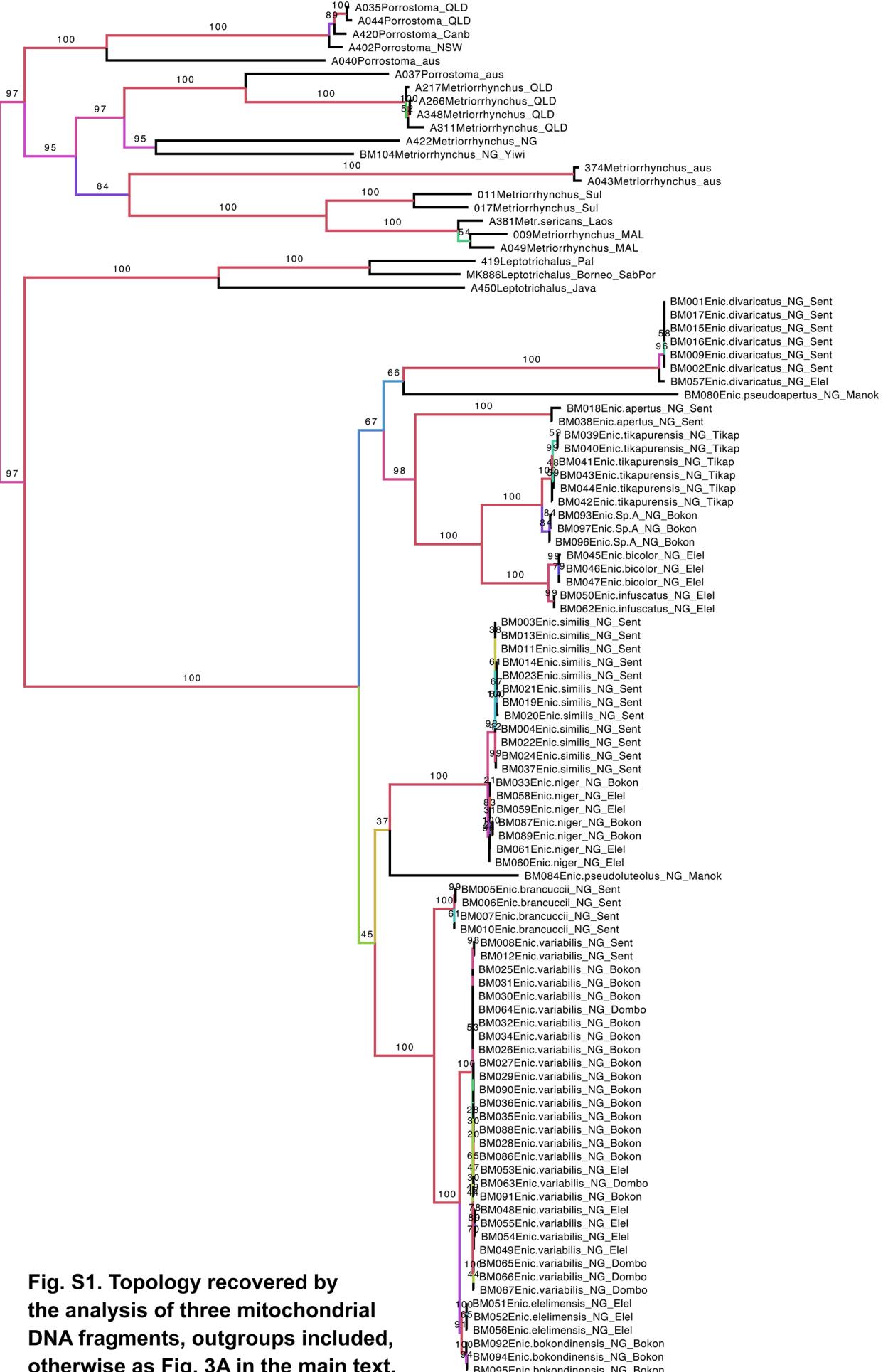


Fig. S1. Topology recovered by the analysis of three mitochondrial DNA fragments, outgroups included, otherwise as Fig. 3A in the main text.

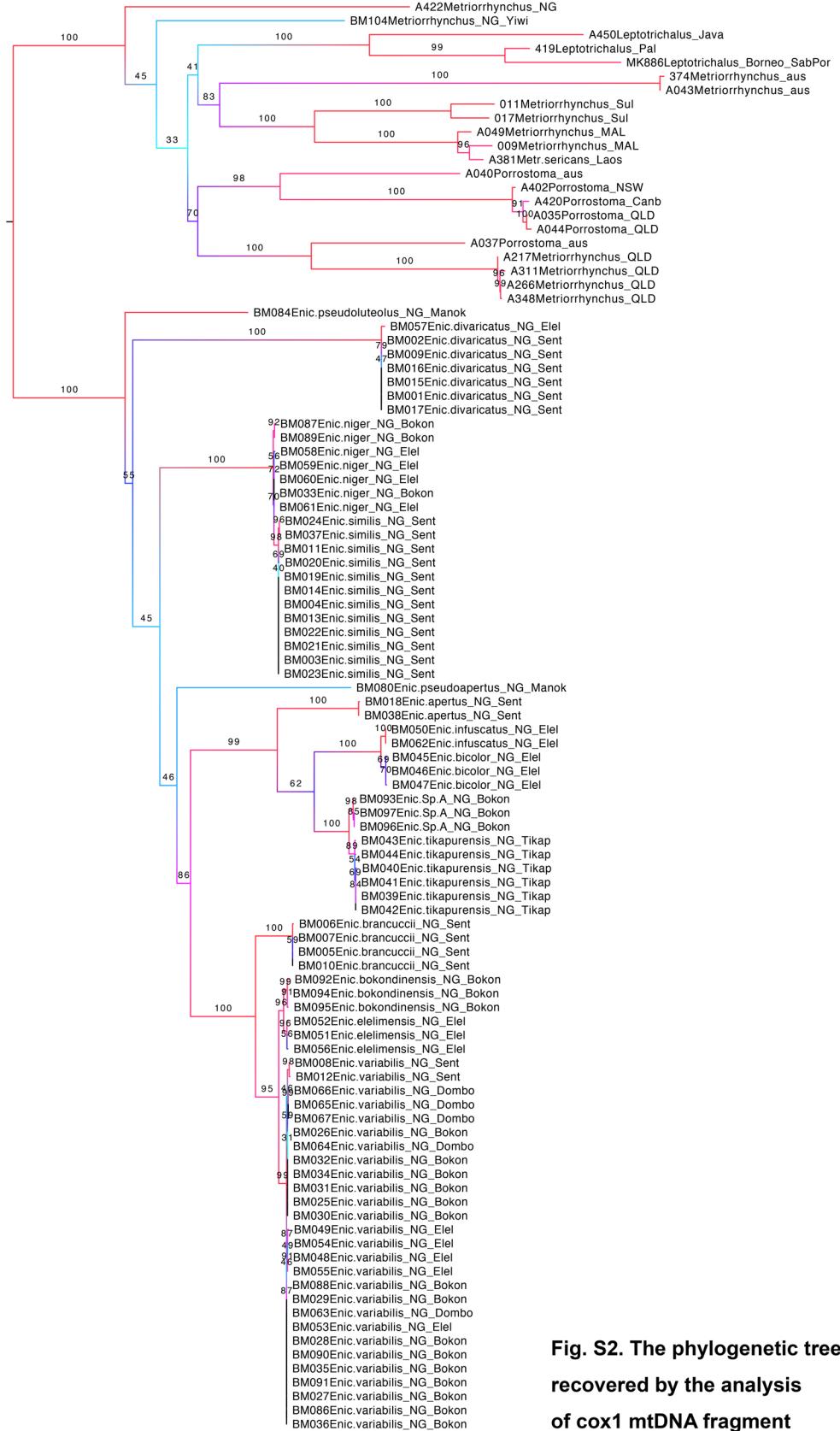


Fig. S2. The phylogenetic tree recovered by the analysis of cox1 mtDNA fragment

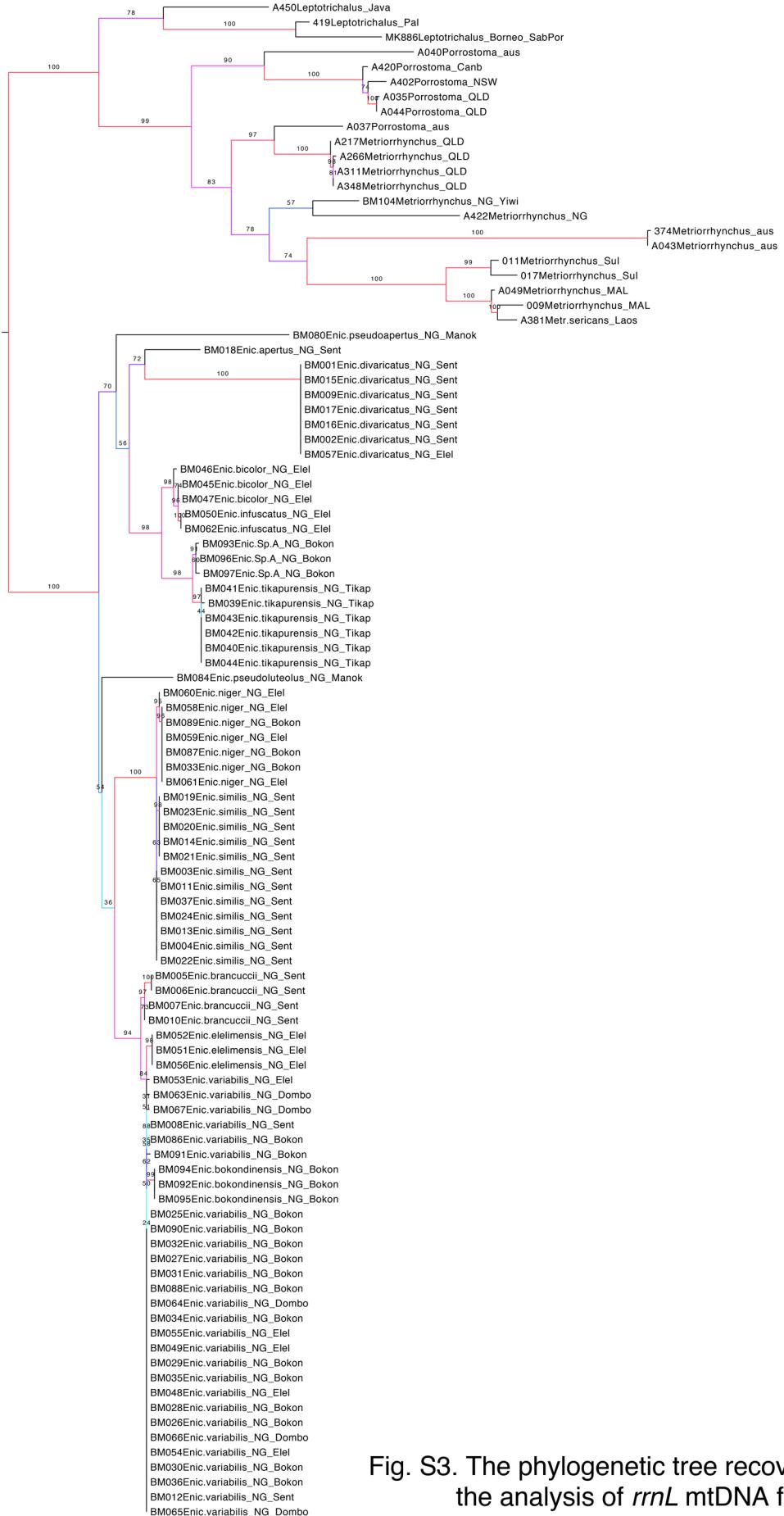


Fig. S3. The phylogenetic tree recovered by the analysis of *rrnL* mtDNA fragment

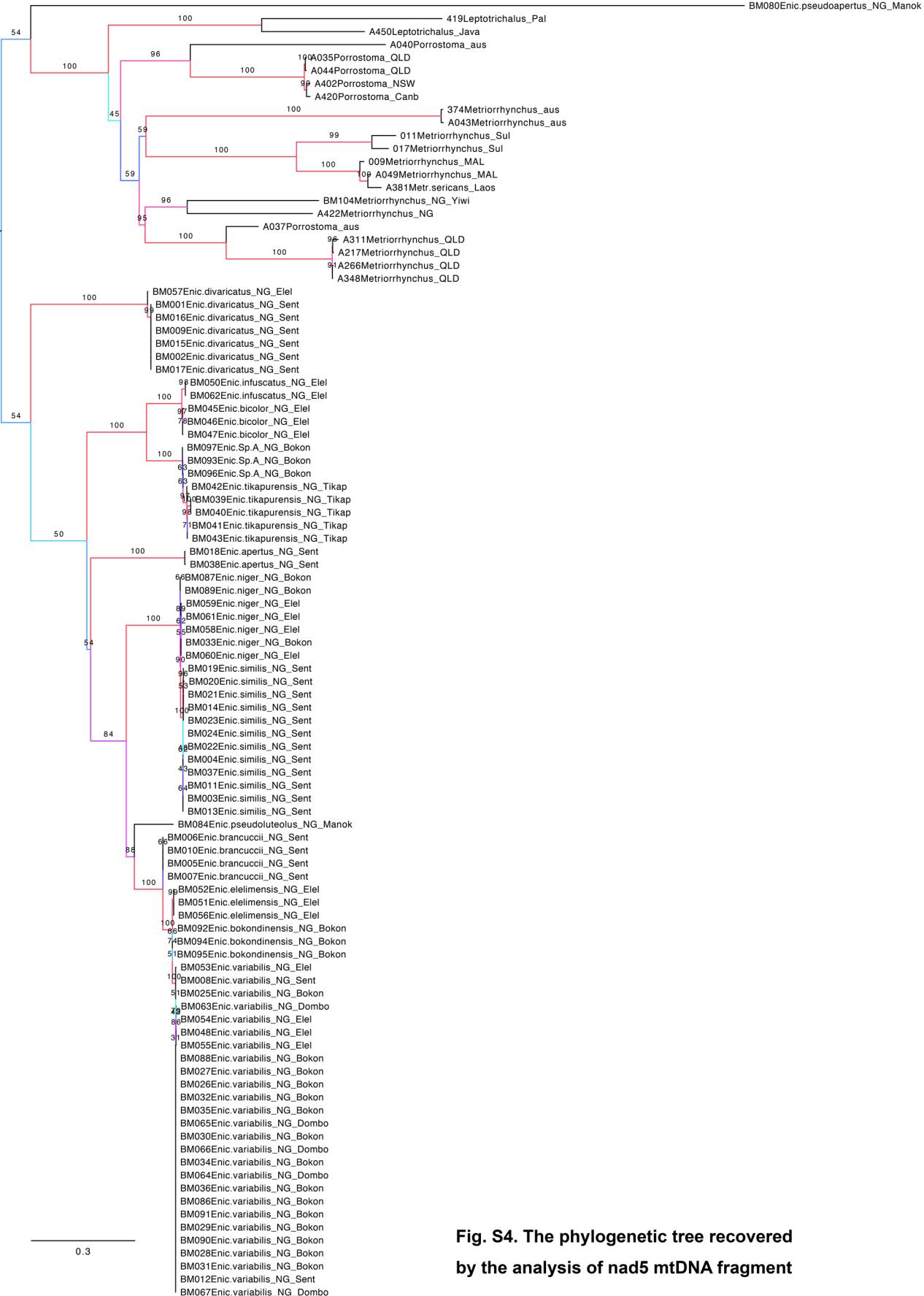


Fig. S4. The phylogenetic tree recovered by the analysis of nad5 mtDNA fragment

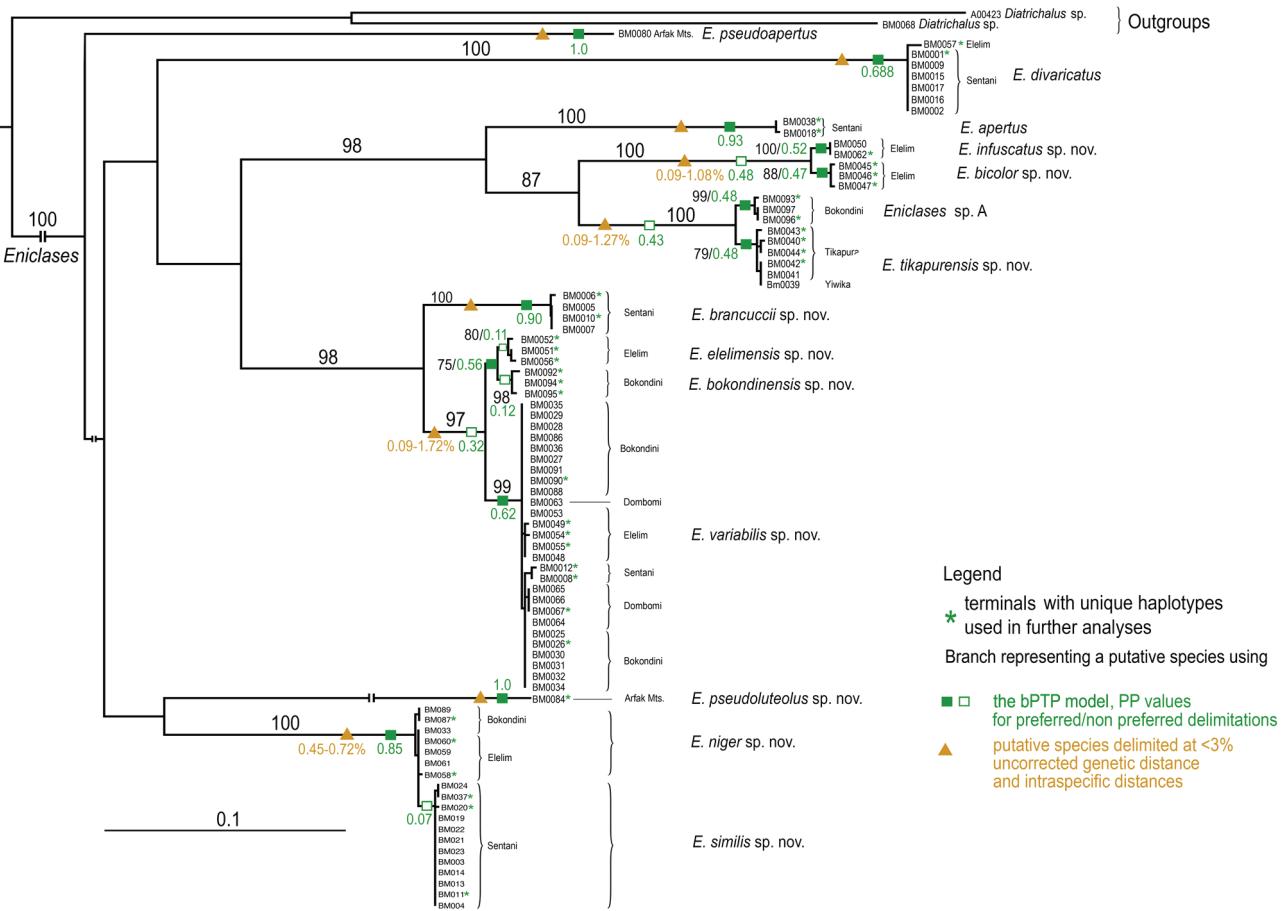


Figure S5. Bayesian Poisson Tree Process species delimitation using cox1 mtDNA data (reprinted from Bocek & Bocak 2016)