

SYSTEMATIC POSITION OF DINIDORIDAE AND TESSARATOMIDAE WITHIN THE SUPERFAMILY PENTATOMOIDEA (HEMIPTERA: HETEROPTERA) BASED ON THE ANALYSIS OF THE MITOCHONDRIAL CYTOCHROME OXIDASE II SEQUENCES

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ABSTRACT: Systematic position of two families, i.e., Dinidoridae and Tessaratomidae, usually regarded as sister groups within Pentatomoidea are analyzed using sequences of the mitochondrial cytochrome oxidase II. Results of the present study stay in contrary to the results of previous molecular analyses suggesting a close affinity of Dinidoridae and Tessaratomidae.

KEY WORDS: Hemiptera, Heteroptera, Dinidoridae, Tessaratomidae, molecular phylogeny, cytochrome oxidase II.

Introduction

The systematic position of Dinidoridae and Tessaratomidae, two pentatomoid families, has been infrequently studied, so far. Twice, the analyses were based on morphological characters (Gapud 1991, Kment and Vilímová 2010); the other six were based on nuclear and mitochondrial DNA sequences analyses (Xie et al. 2005, Li et al. 2005, 2006a, b, Lis et al. 2012, Tian et al. 2011).

Results of almost all those studies suggested a close affinity of both families, usually identifying the Dinidoridae, as a sister group of Tessaratomidae. Additionally, Grazia et al.

(2008) in the study based on a combined set of morphological data and DNA sequences, presented a similar proposition.

In this paper we present results of our analyses based on sequences of the cytochrome oxidase II, the mitochondrial subunit never before utilized for resolving the relationships within the entire superfamily Pentatomoidea.

Material and methods

Species used in this study are listed in Table 1. In total, 28 species representing 6 pentatomoid families, and two species representing an outgroup, were analyzed.

All sequences of the cytochrome oxidase II (COII) for the studied species were obtained from GenBank. Sequence alignments were performed with Clustal W (using default settings) in MEGA 4.0.2 software (Tamura et al. 2007, Kumar et al. 2008).

Nucleotide composition and substitution patterns were calculated in MEGA 4.0.2 (Tamura et al. 2007, Kumar et al. 2008). The overall transition / transversion bias (R) was calculated for each data set, where $R = [A*G* k_1 + T*C* k_2] / [(A+G) * (T+C)]$ (Tamura et al. 2004).

Phylogenetic analyses using neighbor-joining (NJ), maximum parsimony (MP), minimum evolution (ME), and maximum likelihood (ML) methods were carried out using the MEGA software version 5.10 (Tamura et al. 2011).

The maximum composite likelihood (Tamura et al. 2004) model of nucleotide substitution was selected for the estimate evolutionary distances for NJ and ME models. Maximum parsimony (MP) analysis was performed using heuristic search procedure (Subtree-Pruning-Regrafting [SPR] search level 3) with 10 replications in Random Additions method for initial tree. Parsimony analysis of the COII dataset identified two most parsimonious trees. Maximum Likelihood (ML) analyses were performed using the heuristic search procedure (Nearest-Neighbor-Interchange NNJ), with initial tree generated automatically by applying NJ and BIONJ algorithms. The GTR model was selected as best-fitting nucleotide substitution model for COII data set. The reliability of the clustering pattern in all trees was determined by the bootstrap test, with 500 replications. All positions containing gaps were omitted from the dataset by using complete-deletion option.

Bayesian inferences (BI) trees were obtained with MrBayes v. 3.2 (Ronquist et al. 2012) using the Markov Chain Monte Carlo technique (MCMC). The evolutionary model was set to GTR + I + Γ (nst=6), and DNA model was set to Codon. Each run lasted for 500,000 generations with a sampling frequency every 1000th generation. After 500,000 generations the standard deviation of split frequency was <0.02.

Trees for MP, ME, NJ, and MJ were edited using MEGA 5.10 (Tamura et al. 2011), and the BI tree with FigTree 1.3.1 (Rambaut 2009).

Results and discussion

Nucleotide alignment. The datasets consist of 585 base pair for the COII protein-coding sequences, of which 244 sites (41.7%) were conserved, 341 were variable (58.3%) and included 277 parsimony-informative sites, and 64 singleton sites.

Nucleotide composition. Nucleotide content of the COII sequences of 30 species used in this study is shown in Table 2. Nucleotide frequencies of the COII sequence of *Coridius chinensis* (Dinidoridae) differs significantly from these of *Eurostus validus* (Tessaratomidae); a difference in A+T content is also substantial, i.e., 68.2% for *E. validus*, and 73.5% for *C. chinensis* (with 71.3% as average for Pentatomoidea).

Nucleotide substitutions (Table 3). There were a total of 570 positions in the final dataset. The nucleotide frequencies are 0.398 (A), 0.318 (T), 0.16 (C), and 0.124 (G). The transition / transversion rate ratios are $k_1 = 1.493$ (purines) and $k_2 = 3.2$ (pyrimidines). The overall transition / transversion bias is $R = 0.646$.

Phylogenetic analyses. All analyses resulted in well resolved phylogenetic trees (Figs 1-5). All trees identified *C. chinensis* (Dinidoridae) as a sister group of the clade consisting of all other pentatomoid taxa (including *E. validus* of Tessaratomidae). Within this clade, *E. validus* (Tessaratomidae) was shown to be related to *Megacocta cribraria* of Plataspidae (ML, MP), or *Fromundus pygmaeus* of Cydnidae (NJ, ME), or even regarded as a sister group to its all other taxa (BI). Nevertheless, what is most important, *E. validus* (Tessaratomidae) was never treated as a sister taxon of *C. chinensis* (Dinidoridae), what stays in contrary to results of all previous molecular analyses suggesting a close affinity of Dinidoridae and Tessaratomidae. Because our analyses included only a single species for each of them, real phylogenetic relationships between these two families remain an open question.

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Table 1. Details of species used in comparative and phylogenetic analyses.

Family	Species	Geographic origin	GenBank (NCBI) accession number	Source
CYDNIDAE	<i>Fromundus pygmaeus</i> (Dallas)	China	AY743595	GenBank (Bu & Zheng, 2004; unpublished) [as <i>Geotomus pygmaeus</i> (Dallas)]
DINIDORIDAE	<i>Macroscytus gibbulus</i> (Ellenrieder)	China	EU427338	Hua et al. 2008 [as <i>M. subaeneus</i> (Dallas)]
PENTATOMIDAE	<i>Coridius chinensis</i> (Dallas)	China	JQ739179	Liu et al. 2012
	<i>Arma custos</i> (Fabricius)	China	AY675192	Bu et al. 2005
	<i>Carbula sinica</i> Hsiao & Cheng	China	AY679134	Bu et al. 2006
	<i>Carbula humerigera</i> (Uhler)	China	AY679133	Bu et al. 2006 [as <i>C. obtusangula</i> Reuter]
	<i>Dybowskyia reticulata</i> (Dallas)	China	AY820270	Bu et al. 2006
	<i>Eurydema dominulus</i> (Scopoli)	China	AY679132	GenBank (Bu & Zheng, 2004; unpublished)
	<i>Eurydema gebleri</i> Kolenati	China	AY679136	GenBank (Bu & Zheng, 2004; unpublished)
	<i>Gonopsis coccinea</i> (Walker)	China	AY820272	Bu et al. 2006
	<i>Graphosoma rubrolineatum</i> (Westwood)	China	AY743594	Bu et al. 2006
	<i>Halyomorpha halys</i> (Stål)	Korea	FJ685650	Lee et al. 2009
	<i>Megarrhamphus hastatus</i> (Fabricius)	China	AY820273	Bu et al. 2006
	<i>Nezara viridula</i> (Linnaeus)	China	EF208087	Hua et al. 2008
	<i>Pentatoma illuminata</i> (Distant)	China	AY675190	Bu et al. 2005
	<i>Pentatoma japonica</i> (Distant)	China	AY675186	Bu et al. 2005
	<i>Pentatoma metallifera</i> (Motschulsky)	China	AY675189	Bu et al. 2005
	<i>Pentatoma rufipes</i> (Linnaeus)	China	AY675188	Bu et al. 2005
	<i>Pentatoma semiannulata</i> (Motschulsky)	China	AY675187	Bu et al. 2005
	<i>Picromerus griseus</i> (Dallas)	China	AY820275	Bu et al. 2006
	<i>Pinthaeus humeralis</i> (Horváth)	China	AY675191	Bu et al. 2005
	<i>Plautia stali</i> Scott	China	AY679137	GenBank (Bu & Zheng, 2004; unpublished)
	<i>Scotinophara horvathi</i> Distant	China	AY820274	GenBank (Bu & Zheng, 2004; unpublished)
PLATASPIDAE	<i>Coptosoma bifarium</i> (Montandon)	China	EU427334	Hua et al. 2008 [under the name <i>C. bifaria</i>]
	<i>Megacopta cribraria</i> (Fabricius)	USA (introduction)	JF288758	GenBank (Eaton & Jenkins; Submitted 02-FEB-2011; data on this specimen published in Jenkins et al. 2010)
SCUTELLERIDAE	<i>Poecilocoris lewisi</i> (Distant)	China	AY820271	Bu et al. 2006
	<i>Poecilocoris nepalensis</i> (Herrich-Schaeffer)	China	AY820269	Bu et al. 2006
TESSARATOMIDAE	<i>Eurostus validus</i> Dallas	China	AY820268	GenBank (Bu & Zheng, 2004; unpublished)
COREOIDEA (OUTGROUP)	<i>Hydaropsis longirostris</i> (Hsiao)	China	EU427337	Hua et al. 2008
	<i>Rhopalus latus</i> (Jakovlev)	China	EU427333	Hua et al. 2008 [as <i>Aeshyntelus notatus</i> Hsiao]

Table 2. Nucleotide content of the COII sequences of species used in this study.

Taxon	T	C	A	G
<i>Fromundus pygmaeus</i> (CYDNIDAE)	30.6	17.6	40.5	11.3
<i>Macroscytus gibbulus</i> (CYDNIDAE)	30.9	16.6	40.0	12.5
<i>Gonopsis coccinea</i> (PENTATOMIDAE)	31.5	15.0	42.6	10.9
<i>Megarrhamphus hastatus</i> (PENTATOMIDAE)	30.9	15.2	42.1	11.8
<i>Dybowskyia reticulata</i> (PENTATOMIDAE)	27.0	19.0	41.5	12.5
<i>Graphosoma rubrolineatum</i> (PENTATOMIDAE)	29.1	17.8	40.3	12.8
<i>Scotinophara horvathi</i> (PENTATOMIDAE)	31.6	17.2	37.9	13.3
<i>Arma custos</i> (PENTATOMIDAE)	31.1	16.4	39.0	13.5
<i>Picromerus griseus</i> (PENTATOMIDAE)	30.3	17.4	40.0	12.3
<i>Pinthaeus humeralis</i> (PENTATOMIDAE)	29.9	17.8	40.0	12.3
<i>Carbula humerigera</i> (PENTATOMIDAE)	33.3	14.9	39.1	12.6
<i>Carbula sinica</i> (PENTATOMIDAE)	35.6	13.7	37.8	13.0
<i>Eurydema dominulus</i> (PENTATOMIDAE)	30.8	16.4	40.0	12.8
<i>Eurydema gebleri</i> (PENTATOMIDAE)	31.5	16.4	39.8	12.3
<i>Halyomorpha halys</i> (PENTATOMIDAE)	31.6	15.2	40.0	13.2
<i>Nezara viridula</i> (PENTATOMIDAE)	31.6	16.2	39.5	12.6
<i>Pentatoma illuminata</i> (PENTATOMIDAE)	34.0	13.8	40.5	11.6
<i>Pentatoma japonica</i> (PENTATOMIDAE)	32.6	15.4	40.2	11.8
<i>Pentatoma metallifera</i> (PENTATOMIDAE)	33.7	13.5	39.1	13.7
<i>Pentatoma rufipes</i> (PENTATOMIDAE)	32.5	15.9	39.0	12.6
<i>Pentatoma semiannulata</i> (PENTATOMIDAE)	33.5	14.5	39.8	12.1
<i>Plautia stali</i> (PENTATOMIDAE)	33.7	15.0	38.6	12.6
<i>Coptosoma bifarium</i> (PLATASPIDAE)	31.3	17.4	37.3	14.0
<i>Megacopta cribraria</i> (PLATASPIDAE)	28.2	19.7	38.6	13.5
<i>Poecilocoris lewisi</i> (SCUTELLERIDAE)	31.1	16.8	39.7	12.5
<i>Poecilocoris nepalensis</i> (SCUTELLERIDAE)	33.5	14.9	39.3	12.3
<i>Eurostus validus</i> (TESSARATOMIDAE)	29.1	17.4	39.1	14.4
<i>Coridius chinensis</i> (DINIDORIDAE)	32.5	15.0	41.0	11.5
Average for Pentatomoidea	31.5	16.2	39.7	12.6
<i>Hydaropsis longirostris</i> (COREOIDEA: Coreidae)	38.6	14.9	34.5	12.0
<i>Rhopalus latus</i> (COREOIDEA: Rhopalidae)	33.8	16.1	37.6	12.5
Average for Pentatomomorpha	31.8	16.1	39.5	12.6

Table 3. Frequency of nucleotide substitutions in the COII protein-coding sequences among 30 species. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

	A	T	C	G
A	-	<i>7.39</i>	<i>3.72</i>	4.28
T	<i>9.23</i>	-	11.89	<i>2.87</i>
C	<i>9.23</i>	23.64	-	<i>2.87</i>
G	13.78	<i>7.39</i>	<i>3.72</i>	-

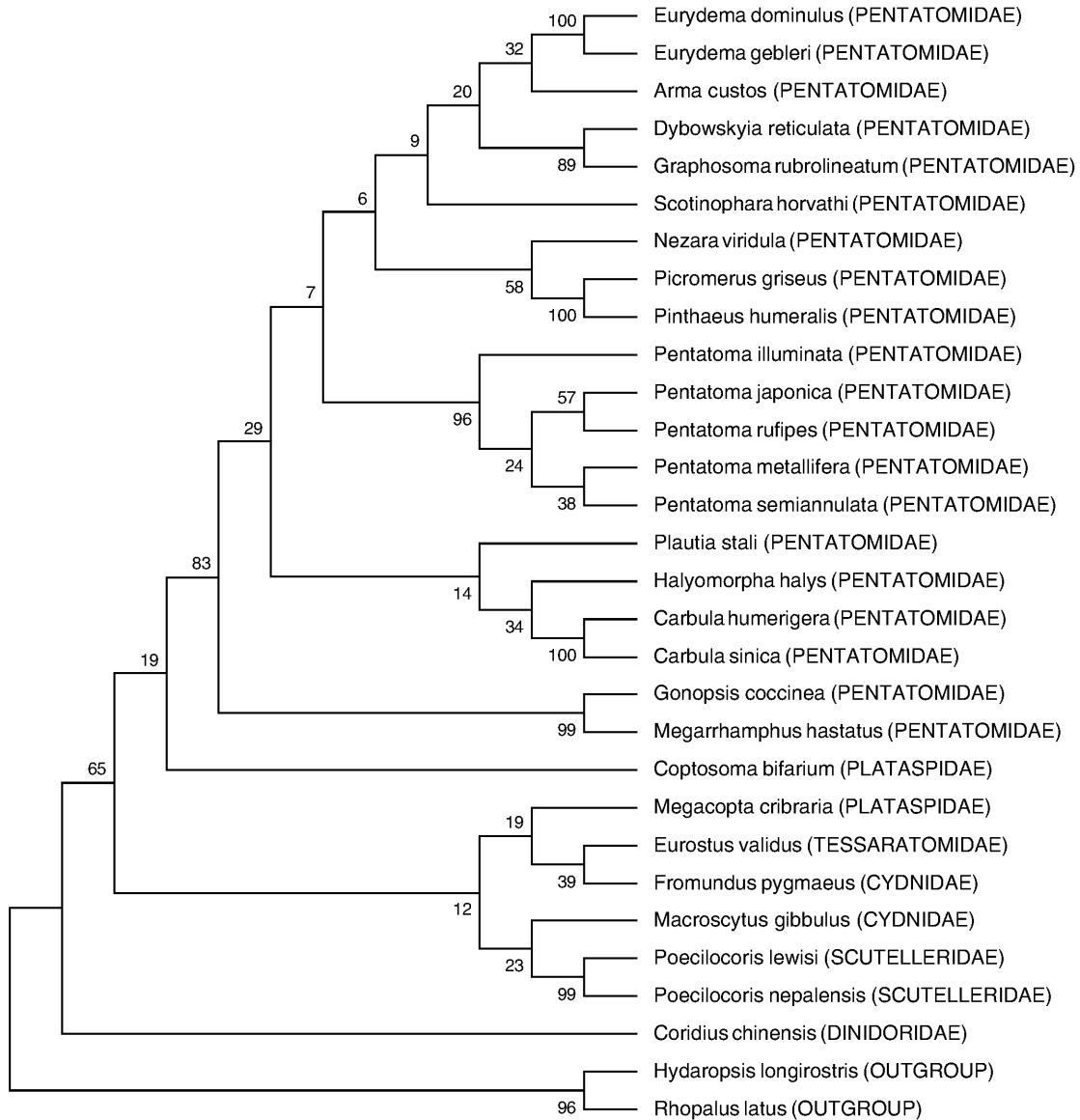


Fig. 1. Phylogenetic tree generated by neighbor-joining (NJ) analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

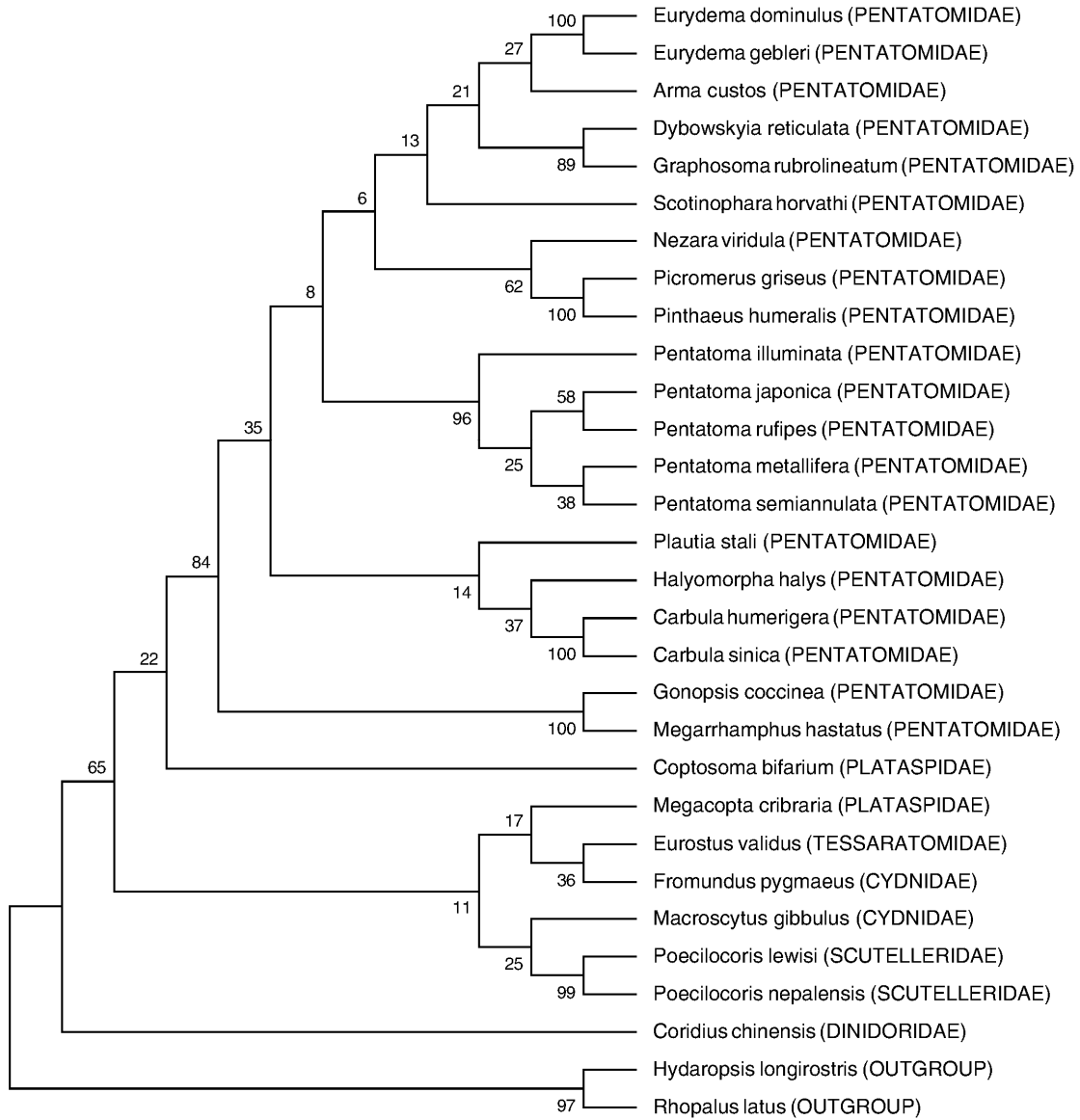


Fig. 2. Phylogenetic tree generated by minimum evolution (ME) analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

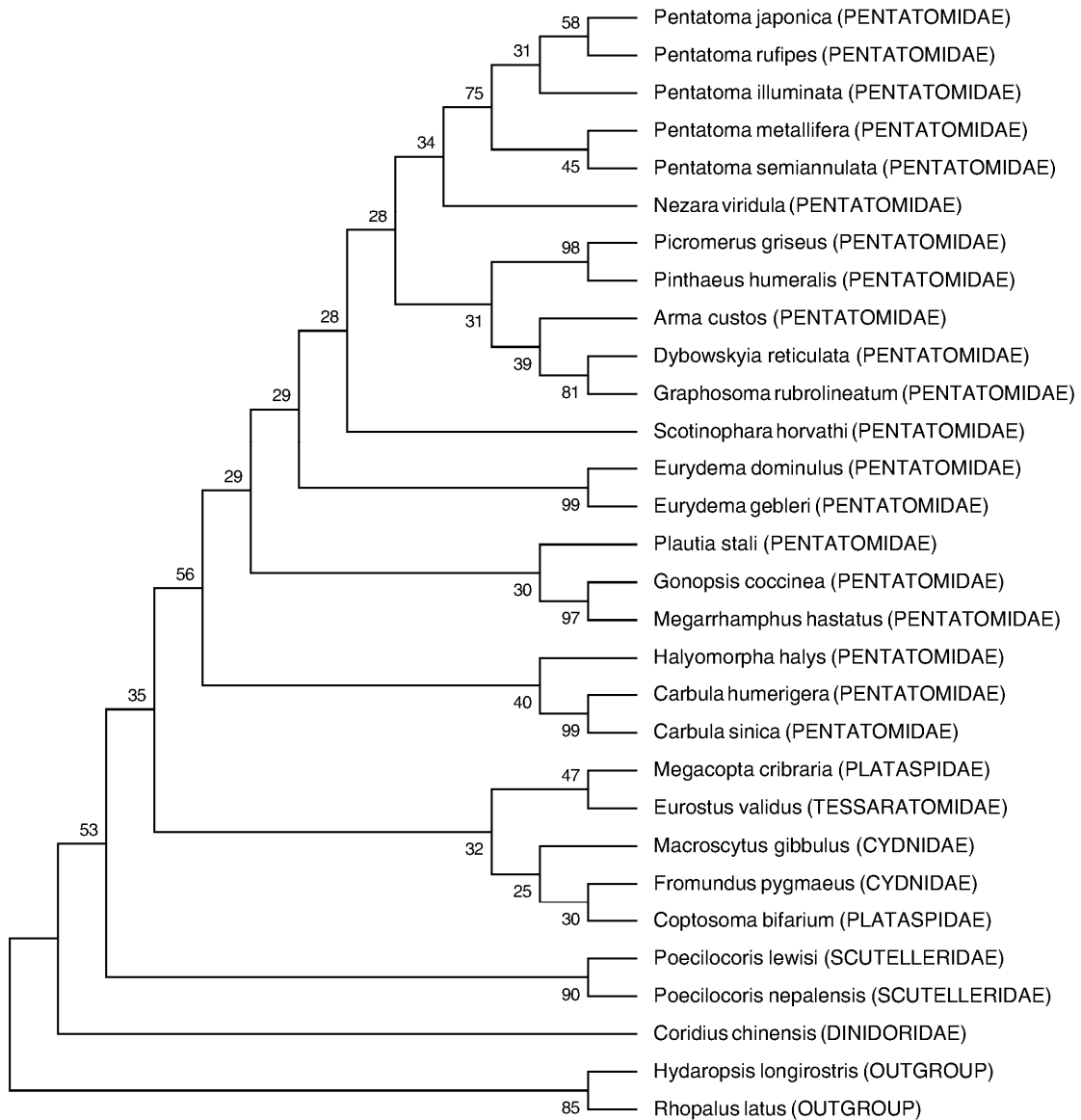


Fig. 3. Phylogenetic tree generated by maximum parsimony (MP) analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

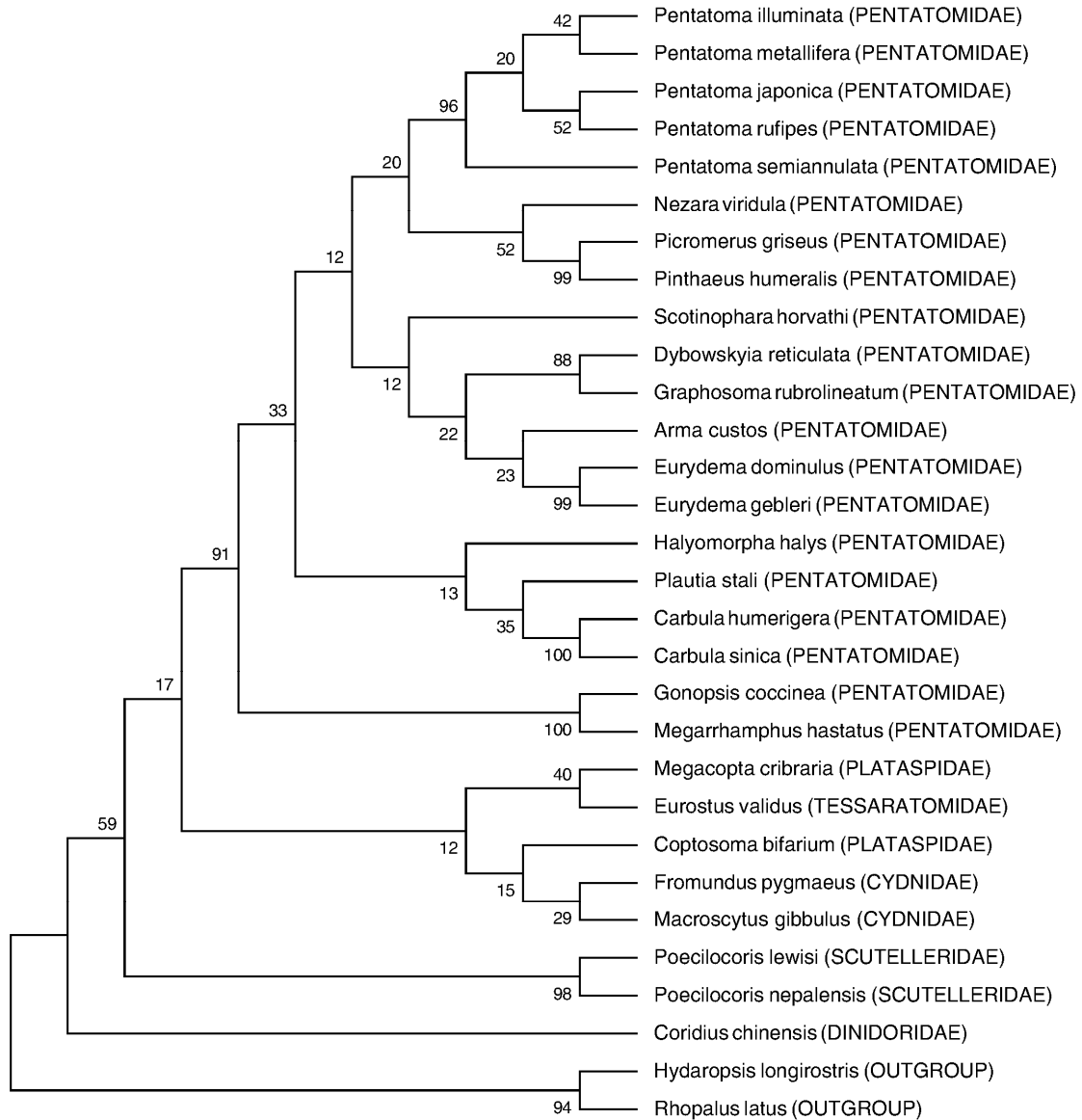


Fig. 4. Phylogenetic tree generated by maximum likelihood (ML) analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

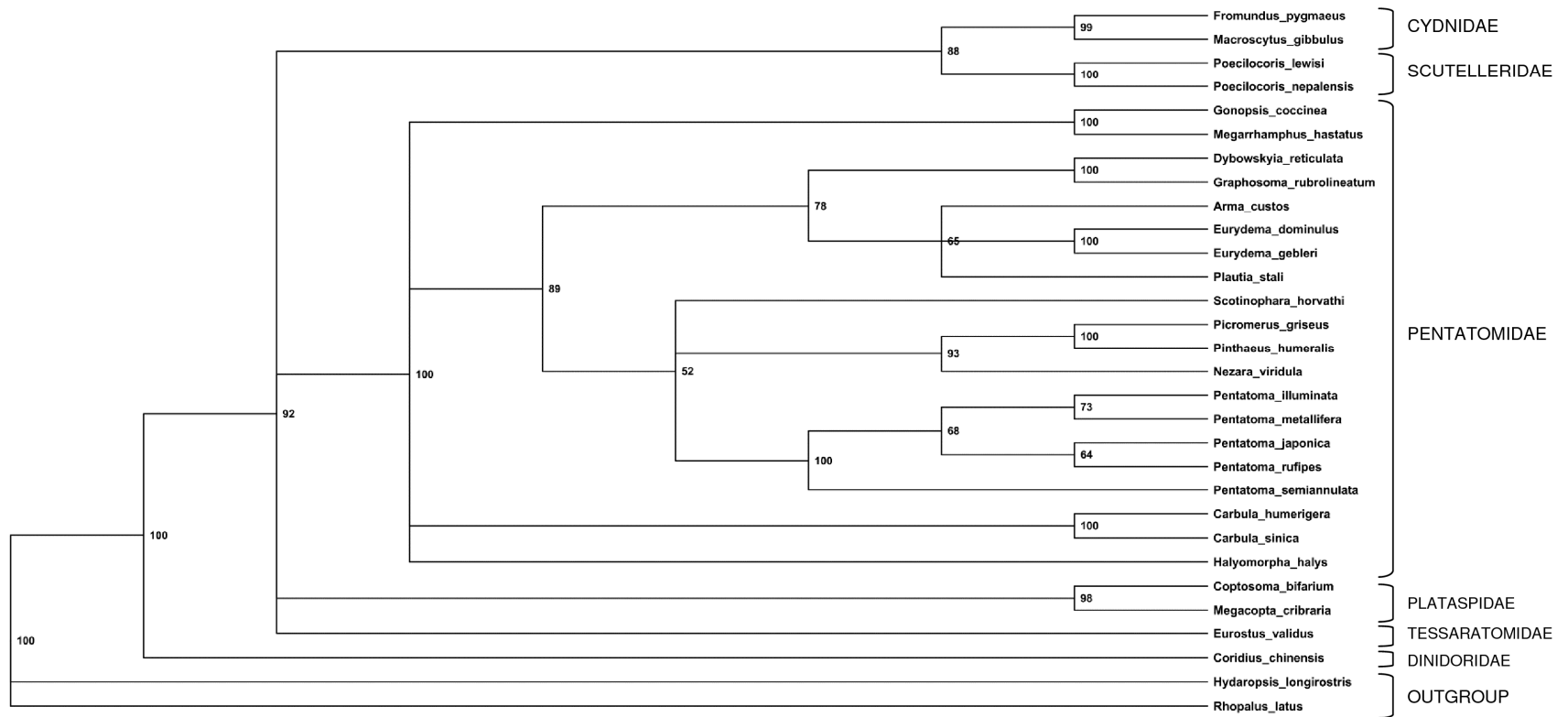


Fig. 5. Phylogenetic tree obtained from the Bayesian inference analyses (posterior probabilities are indicated at each node).