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Evaluation of the usefulness of the mitochondrial DNA control region for resolving phylogenetic relationships at different taxonomic levels in Hemiptera

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Abstract: The usefulness of the mitochondrial (mt) DNA control region (CR) for resolving phylogenetic relationships at different taxonomic levels within the order Hemiptera was estimated. The analyses suggest that the mtDNA control region can be useful as a molecular marker in studies at lower taxonomic levels (i.e., within families); however, at higher levels (i.e., within infraorders, suborders, or within the entire order), it might not reflect true phylogenetic relationships, as shown by studies on the complete mitogenomes.

Key words: Hemiptera, Heteroptera, Cicadomorpha, Fulgoromorpha, mtDNA, control region, molecular marker, phylogeny.

Introduction

The control region (CR) is the only non-coding fragment in the mitochondrial genome of insects, and it is most probably involved in the regulation of transcription and the control of DNA replication (Zhang & Hewitt 1997). A high content of adenosine (A) and thymine (T) nucleotides in the CR (usually higher than 85%) is common to all insects, and therefore, the control region is also known as an "A+T-rich region" (Zhang & Hewitt 1997).

In contrast to vertebrates, the location of the control region among invertebrates is more variable, presumably due to tRNA transposition during evolution. The size of the CR also varies greatly, and differences can be found even between closely related taxa. Such variation is caused by the tandem repeats, and these duplications have been observed in most of the insect CRs studied to date, which strongly suggests convergent evolution at higher taxonomic levels (Zhang & Hewitt 1997).

Taking the structure of the CR into consideration, insects can be divided into two groups (Zhang & Hewitt 1997):

(1) the *Drosophila* species group, where the CR contains two different domains, i.e., one highly conserved domain, which is flanked on one side by $tRNA^{Ile}$ (this is probably involved in the replication process), and the other domain, which is highly variable in nucleotide sequence and in length; moreover, the tandem repeats were observed to occur in both domains;

(2) the group consisting of other insects, in which these two domains cannot be recognised. Instead, these insects contain conserved sequence blocks scattered throughout the whole region, and the tandem repeats were also observed throughout the entire domain.

However, most importantly, these five structural elements can be distinguished in the insect CR (Zhang & Hewitt 1997): (1) a poly-thymidine stretch located at the 5´-end of the CR, near to the $tRNA^{Ile}$ gene, and presumably involved in the control of transcription and/or replication initiation; (2) a [TA(A)]n-like stretch located mostly between the poly-thymidine stretch and the stem–loop; (3) the highly conserved stem–loop structure probably involved in the second-strand replication process; (4) 'TATA' (5´) and '$G(A)_nT$' (3´) sequences associated with the stem–loop structure; and (5) a block of guanine- (G) and A-rich sequences.

The structure of the mitochondrial CR in different species of Hemiptera is summarised in Tables 1–11, its varying size is provided in Tables 12–13, and differences in the flanking CR are shown in Tables 14–15 (usually these are srRNA and $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$ genes, but there are exceptions, e.g., *Stenopirates* sp., in

which the CR is adjacent to the genes tRNAS2 and lrRNA, or *Nabis apicalis*, in which the CR is flanked by srRNA and ND2). A high content of A and T nucleotides (Table 16), which is a characteristic of insects, is also common to Hemiptera (Hua et al. 2008; Liu et al. 2012; Shi et al. 2012; Song et al. 2013).

Recently, features and structures of hemipteran mitogenomes and their implications for phylogeny were reviewed by Wang et al. (2015). However, the usefulness of the sole mitochondrial DNA CR for phylogenetic considerations in Hemiptera has not been evaluated; therefore, we aimed to analyse whether the non-coding fragment in the mitogenome is a good molecular marker for resolving phylogenetic problems in Hemiptera.

Material and methods

Analysed taxa. For the analyses, taxa representing different taxonomic levels were selected (Appendix 1); namely, two families (i.e., Nabidae, Reduviidae), three infraorders (i.e., Nepomorpha, Pentatomomorpha, Cimicomorpha), three suborders (i.e., Cicadomorpha, Fulgoromorpha, Heteroptera), as well as the entire order Hemiptera. Division of the order into suborders follows Wang et al. (2015). The sources for the species photos used in the phylograms were provided in the Appendix 2.

CR sequence acquisition. The CR sequences for study were initially searched for in the GenBank databases, but because no sequences were found, 48 complete genomes of the species representing the insect order Hemiptera were selected, which were deposited in GenBank (Appendix 1). Subsequently, the CR sequence was transferred into FASTA format.

Sequence alignments. All the CR sequences that were downloaded from GenBank in FASTA format were aligned using the default parameters of ClustalW (Larkin et al. 2007) implemented in MEGA v.6.0 (Tamura et al. 2013).

Phylogenetic analyses. Analyses using the neighbour-joining (NJ), minimum evolution (ME), maximum parsimony (MP), and maximum likelihood (ML) methods were performed using MEGA 6.0 (Tamura et al. 2013). All trees were edited using MEGA 6.0 (op. cit.). The outgroups were selected as follows: *Lygus lineolaris* (Pal. de Beauv.) and *Nesidiocoris tenuis* (Reut.) (Miridae) for the analysis within the Nabidae and Reduviidae; *Dolycoris baccarum* (L.) and *Nezara viridula* (L.) (Pentatomidae) for the analysis within the infraorder Cimicomorpha; *Aquarius paludum* (F.) (Gerridae) for the analysis within the infraorder Nepomorpha; *Gorpis annulatus* Paiva and *G. humeralis* (Dist.) (Nabidae) for the analysis within the infraorder Pentatomomorpha; *Hackeriella veitchi* (Hacker) (Peloridiidae) for the analysis within the suborder Cicadomorpha and Heteroptera; *Callitettix versicolor* (F.) (Cercopidae) for the analysis within the suborder Fulgoromorpha; and *Psococerastis albimaculata* Li & Yang (Psocoptera: Psocomorpha) for the analysis within the entire order Hemiptera.

Results and discussion

The results of analyses are presented from the family level to the order level.

Family Nabidae (Hemiptera: Heteroptera: Cimicomorpha). Figures 1–4 present the phylogenetic relationships among the species of the family Nabidae. The bootstrap values are very high both in the outgroups, as well as for species of the genus *Gorpis* and the *Himacerus*. The highest scores were obtained using the ML method. The topology of the tree was identical for all methods used (Figs. 1–4). In the resulting trees, the genus *Himacerus* was shown to be more closely related to the genus *Gorpis* than to *Nabis apicalis*, which is in contrast to recent results based on the analysis of the complete mitogenomes of Hemiptera (Li et al. 2012b; Wang et al. 2015), because, as shown in Fig. 5, *Gorpis annulatus* and *G. humeralis* should be placed within the tribe Gorpini, whereas the other species, i.e., *Himacerus apterus*, *H. nodipes* and *Nabis apicalis* belong to to the tribe Nabini.

Family Reduviidae (Hemiptera: Heteroptera: Cimicomorpha). Figures 6–9 show the phylogenetic relationships among species of the family Reduviidae resulting from our analyses; all these relationships are supported by relatively high bootstrap values. Most importantly, our results (similar to those for the family Nabidae), contrast with the present taxonomic position of the studied taxa, i.e., *Triatoma dimidiata* (Triatominae) was shown to be most closely related to *Agriosphodrus dohrni* (Harpactorinae) when the ML, NJ, and ME methods were used, but in the MP analysis, *A. dohrni* showed the closest relationship to *Valentia hoffmanni* (Salyavatinae). However, when the recent classification of the family is taken into consideration (Weirauch & Munro 2009; Li et al. 2011), *V. hoffmanni* (subfamily Salyavatinae) should be closely related to *T. dimidiata* (subfamily Triatominae), and only then to *A. dohrni* (subfamily Harpactorinae).

Infraorder Cimicomorpha (Hemiptera: Heteroptera). Figures 10–13 present the phylogenetic trees of the infraorder Cimicomorpha (including the Miridae, Nabidae, and Reduviidae) obtained from our analyses. The bootstrap values are relatively high for each family level, and the tree topology is the same irrespective of the used method. Close relationships were found for species of the family Reduviidae and those in the fami-

ly Nabidae. However, the family Miridae appears to be polyphyletic according to our results, because the analysed species are scattered throughout the tree; i.e., *Nesidiocoris tenuis* is a sister group to all species of the family Nabidae, whereas *Lygus lineolaris* was identified as a sister species to all studied taxa of the Nabidae and Reduviidae, and also to the mirid *N. tenuis*.

Infraorder Nepomorpha (Hemiptera: Heteroptera). Figures 14–17 present the phylogenetic trees of the infraorder Nepomorpha. For the ML, NJ and ME methods, the bootstrap values are high, but are much lower for the MP method. The tree topology is identical for all employed methods and is consistent with the current phylogenetic reconstruction of the Nepomorpha when the complete mitogenomes were used (Hua et al. 2009).

Infraorder Pentatomomorpha (Hemiptera: Heteroptera). Figures 18–21 present the phylogenetic trees of the infraorder Pentatomomorpha. Significant differences in the topology of the trees exist, depending on the employed method; however, the bootstrap values for all trees are relatively low, therefore, all results should be regarded with caution, especially those that suggest polyphyly of the Aradidae and Plataspidae families.

Suborder Cicadomorpha (Hemiptera). Figures 22–25 present the phylogenetic trees of the suborder Cicadomorpha. The tree topology is identical for all used methods. In all trees, *Paphnutius ruficeps* (Cercopidae) was identified to be more closely related to *Philaenus spumarius* (Aphrophoridae) than to other species of the Cercopidae.

Suborder Fulgoromorpha (Hemiptera). The phylogenetic trees of species in the suborder Fulgoromorpha are presented in Figures 26–29. The highest bootstrap values were obtained using the MP and ML methods. The tree topology was identical for the ML, NJ and ME methods. When the MP method was used for phylogenetic reconstruction, *Laodelphax striatella* was identified to be more closely related to *Sogatella furcifera* than to *Nilaparvata lugens*. When the tree topology obtained here is compared with the phylogenetic trees produced by Bourgoin et al. (1997) the results are consistent only in one respect: the close relationship between the Flatidae and Ricaniidae families. In contrast, our phylogeny showed that the Fulgoridae family is more closely related to the Issidae family, than to the Flatidae and Ricaniidae, as was shown by Bourgoin et al. (1997). Moreover, the Delphacidae family in our cladograms is more closely related to the Issidae than to the Fulgoridae.

Suborder Heteroptera (Hemiptera). The bootstrap values for all obtained trees are relatively low and their topology is similar for ML, NJ and ME methods (Figs. 30–33). Despite the general similarities between our results and the currently accepted phylogeny (Song et al. 2012), several discrepancies exist between them, e.g., our studies show that *Himacerus nodipes* is more closely related to species of other infraorders than to its congener *H. apterus;* and the Miridae and Aradidae families are presumed to be polyphyletic (which is incompatible with the recent classification of Song et al. (2012)).

Order Hemiptera. The bootstrap values are relatively low for each resulting tree (Figs. 34–37), and differences in tree topology result from the use of different methods. Moreover, according to the produced trees, the suborder Fulgoromorpha is more closely related to Heteroptera than to Cicadomorpha, which disagrees with the current molecular classification of the order (Song et al., 2012; Cui et al. 2013).

Conclusions

The control region of the hemipteran mitochondrial genome, which is the only major non-coding mitochondrial DNA region, has never been evaluated for its efficacy as a molecular marker to resolve phylogenetic problems in this insect order.

Our studies carried out at different taxonomic levels, i.e., family, infraorder, suborder and order, showed that the control region can be a useful molecular marker in studies at lower taxonomic levels, i.e., within families, but only to a certain degree. Our phylograms for both the Nabidae and Reduviidae have high bootstrap values, but only the tree topology for the former family is consistent with its current classification. Moreover, at higher levels (i.e., within infraorders, suborders, or the entire order), the phylogenetic relationships inferred from CR phylograms might not often reflect the true phylogenetic relationships as shown by studies based on complete mitogenomes. Therefore, we conclude that the mitochondrial control region alone is not useful for phylogenomic analyses in Hemiptera and should not be considered as a marker for such analyses.

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Table 1. Structure of *Nesidiocoris tenuis* mitochondrial control region (based on Dai et al. 2012)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Cimicomorpha	Miridae	*Nesidiocoris tenuis*
CR length	**Flanking**		**Structural elements**	
3 155 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$		three sections: **1** – one type I repeat unit (100 bp) and two type II repeat units (100 bp; the identity between type I and type II is 99%) **2** – five type III repeat units (60 bp) and six type IV repeat units (60 bp; the identity between type III and type IV is 99%) **3** – three type V repeat units (197 bp), two type VI repeat units (197 bp) and two type VII repeat units (197 bp)	

Table 2. Organization of the control region in nabid mtDNAs (based on Li et al. 2012b)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Cimicomorpha	Nabidae	*Gorpis annulatus* *Gorpis humeralis* *Himacerus apterus* *Himacerus nodipes* *Nabis apicalis*
CR length	**Flanking**		**Structural elements**	
G. annulatus			five sections: **1** – highly variable domain is highly variable both in nucleotide sequence and ength; this domain contains tandem repeats **2** – *stem-loop* structure is highly conserved and probably involved in the second strand replication process **3** – A+T-rich stretch **4** – C-rich stretch **5** – G+A-rich stretch (probably it is taxon-specific)	
1 189 bp	srRNA & $tRNA^{Ile}$			
G. humeralis				
1 367 bp	srRNA & $tRNA^{Ile}$			
H. apterus				
~1 554 bp (nearly complete mitogenome)	srRNA & $tRNA^{Ile}$			
H. nodipes				
~1 247 bp (nearly complete mitogenome)	srRNA & $tRNA^{Ile}$			
N. apicalis				
1 070 bp	srRNA & NADH 2			

Table 3. Structure of *Agriosphodrus dohrni* mitochondrial control region (based on Li et al. 2011)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Cimicomorpha	Reduviidae	*Agriosphodrus dohrni*
CR length	**Flanking**		**Structural elements**	
1 643 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$		five sections: **1** – G+C-rich stretch (410 bp; the guanine and cytosine nucleotides content (43.4%) is higher than the average of the entire genome) **2** – CSB - short conserved sequence block (contains G-element) **3** – A+T-rich stretch (188 bp; the adenine and thymine nucleotides content: 79.2%) **4** – region composed of six tandem repeats **5** – the remainder of the CR	

Table 4. Structure of *Sirthenea flavipes* mitochondrial control region (based on Gao et al. 2013)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Cimicomorpha	Reduviidae	*Sirthenea flavipes*
CR length	**Flanking**		**Structural elements**	
1 295 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$		six sections: **1** – repeat region **2** – G+C-rich stretch (36.3%) **3** – CSB – short conserved sequence block (contains G-element) **4** – A+T-rich stretch (80.2%) **5** – repeat region **6** – the remainder of the CR	

Table 5. Structure of *Triatoma dimidiata* mitochondrial control region (based on Dotson & Beard 2001; Li et al. 2011)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Cimicomorpha	Reduviidae	*Triatoma dimidiata*
CR length	**Flanking**		**Structural elements**	
2 165 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$		five sections: **1** – G+C-rich stretch (450 bp; 40%) **2** – CSB - short conserved sequence block (contains G-element) **3** – A+T-rich stretch (400 bp; 77.5%) **4** – region composed of eight tandem repeats **5** – the remainder of the CR	

Table 6. Structure of *Valentia hoffmanni* mitochondrial control region (based on Hua et al. 2009; Li et al. 2011)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Cimicomorpha	Reduviidae	*Valentia hoffmanni*
CR length	**Flanking**		**Structural elements**	
725 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$		five sections: **1** – G+C-rich stretch **2** – CSB - short conserved sequence block (contains G-element) **3** – A+T-rich stretch **4** – region composed of tandem repeats **5** – the remainder of the CR	

Table 7. Structure of *Stenopirates* sp. mitochondrial control region (based on Li et al. 2012a)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Enicocephalomorpha	Enicocephalidae	*Stenopirates* sp.
CR length	**Flanking**		**Structural elements**	
765 bp	tRNAS2 (tRNAS(UCN)) & lrRNA		five sections: **1** – A+T-rich stretch (29 bp) **2** – G+C-rich stretch (poly-C fragment: 9 bp; poly-G fragment: 14 bp → equivalent of G-element in *T. dimidiata* and *A. dohrni*) **3** – region composed of tandem repeats (441 bp) **4** – *stem-loop* structure **5** – C+T-rich region (102 bp)	

Table 8. Structure of *Aradacanthia heissi* mitochondrial control region (based on Shi et al. 2012)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Pentatomomorpha	Aradidae	*Aradacanthia heissi*
CR length	**Flanking**		**Structural elements**	
1 032 bp	srRNA & $tRNA^{Gln}$-$tRNA^{Ile}$-$tRNA^{Met}$		four sections: **1** – poly-C stretch (10 bp) **2** – *stem-loop* structure (714 bp) **3** – region composed of tandem repeats (272 bp → four 68-bp repeat units) **4** – partial repeat unit (21 bp)	

Table 9. Structure of *Eusthenes cupreus* mitochondrial control region (based on Song et al. 2013)

Order	Suborder	Infraorder	Family	Species
Hemiptera	Heteroptera	Pentatomomorpha	Tessaratomidae	*Eusthenes cupreus*
CR length	**Flanking**		**Structural elements**	
1 520 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$		three sections: **1** – three repetitive sequences (1: 52 bp, 2: 26 bp, 3: 80 bp; they are found in both, the control region and the $tRNA^{Ile}$--$tRNA^{Gln}$-$tRNA^{Met}$-ND2 gene cluster) **2** – three *stem-loop* structures **3** – extra $tRNA^{Gln}$-like sequence (73 bp; it is species-specific)	

Table 10. Structure of *Geisha distinctissima* mitochondrial control region (based on Song & Liang 2009)

Order	Suborder	Family	Species
Hemiptera	Fulgoromorpha	Flatidae	*Geisha distinctis-sima*
CR length	**Flanking**	**Structural elements**	
1 702 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$	five sections: **1** – G+C-rich stretch (38 bp) **2** – the first tandem repeats region (625 bp) **3** – [TA(A)]n-like stretch (739 bp; rich in A+T; includes several poly-A stretches) **4** – the second tandem repeats region (116 bp) **5** – poly-T stretch (11 bp)	

Table 11. Structure of *Sivaloka damnosus* mitochondrial control region (based on Song et al. 2010)

Order	Suborder	Family	Species
Hemiptera	Fulgoromorpha	Issidae	*Sivaloka damnosus*
CR length	**Flanking**	**Structural elements**	
994 bp	srRNA & $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$	five sections: **1** – short [TA(A)]n-like sequence (14 bp) **2** – the first tandem repeats region (367 bp) **3** – [TA(A)]n-like stretch (271 bp; contains several *stem-loop* structures and four poly-T stretches) **4** – the second tandem repeats region (200 bp) **5** – poly-T stretch (10 bp)	

Table 12. Comparison of length of mtDNA control region of selected species of Hemiptera: Heteroptera

Order	Suborder	Infraorder	Family	Species	CR length
Hemiptera	Heteroptera	Cimicomorpha	Miridae	*Lygus lineolaris*	2 318 bp
				Nesidiocoris tenuis	3 155 bp
			Nabidae	*Gorpis annulatus*	1 189 bp
				Gorpis humeralis	1 367 bp
				Himacerus apterus	1 554 bp
				Himacerus nodipes	1 247 bp
				Nabis apicalis	1 070 bp
			Reduviidae	*Agriosphodrus dohrni*	1 643 bp
				Sirthenea flavipes	1 295 bp
				Triatoma dimidiata	2 165 bp
				Valentia hoffmanni	725 bp
		Enicocephalomorpha	Enicocephalidae	*Stenopirates* sp.	765 bp
		Gerromorpha	Gerridae	*Aquarius paludum*	781 bp
		Leptopodomorpha	Saldidae	*Saldula arsenjevi*	697 bp
		Nepomorpha	Naucoridae	*Ilyocoris cimicoides*	609 bp
			Nepidae	*Laccotrephes robustus*	751 bp
			Notonectidae	*Enithares tibialis*	646 bp
			Pleidae	*Paraplea frontalis*	608 bp
		Pentatomomorpha	Alydidae	*Riptortus pedestris*	2 400 bp
			Aradidae	*Aradacanthia heissi*	1 032 bp
				Neuroctenus parus	622 bp
			Berytidae	*Yemmalysus parallelus*	1 181 bp
			Coreidae	*Hydaropsis longirostris*	1 991 bp
			Largidae	*Physopelta gutta*	224 bp
			Malcidae	*Chauliops fallax*	1 148 bp
			Pentatomidae	*Dolycoris baccarum*	1 880 bp
				Nezara viridula	2 190 bp
			Plataspidae	*Coptosoma bifaria*	1 585 bp
				Megacopta cribraria	984 bp
			Pyrrhocoridae	*Dysdercus cingulatus*	1 617 bp
			Rhopalidae	*Stictopleurus subviridis*	685 bp
			Tessaratomidae	*Eusthenes cupreus*	1 520 bp
			Urostylididae	*Urochela quadrinotata*	2 302 bp

Table 13. Comparison of length of mtDNA control region of selected species of Hemiptera: Cicadomorpha, Coleorrhyncha, Fulgoromorpha

Order	Suborder	Family	Species	CR length
Hemiptera	Cicadomorpha	Aphrophoridae	*Philaenus spumarius*	1 835 bp
		Cercopidae	*Abidama producta*	673 bp
			Callitettix versicolor	774 bp
			Paphnutius ruficeps	310 bp
	Coleorrhyncha	Peloridiidae	*Hackeriella veitchi*	1 296 bp
	Fulgoromorpha	Delphacidae	*Laodelphax striatella*	2 042 bp
			Nilaparvata lugens	2 492 bp
			Sogatella furcifera	2 223 bp
		Flatidae	*Geisha distinctissima*	1 702 bp
		Fulgoridae	*Laternaria candelaria*	1 592 bp
			Lycorma delicatula	1 642 bp
		Issidae	*Sivaloka damnosus*	994 bp
		Ricaniidae	*Ricania marginalis*	1 324 bp

Table 14. Comparison of the genes adjacent to the mitochondrial control region of selected species of Hemiptera: Heteroptera

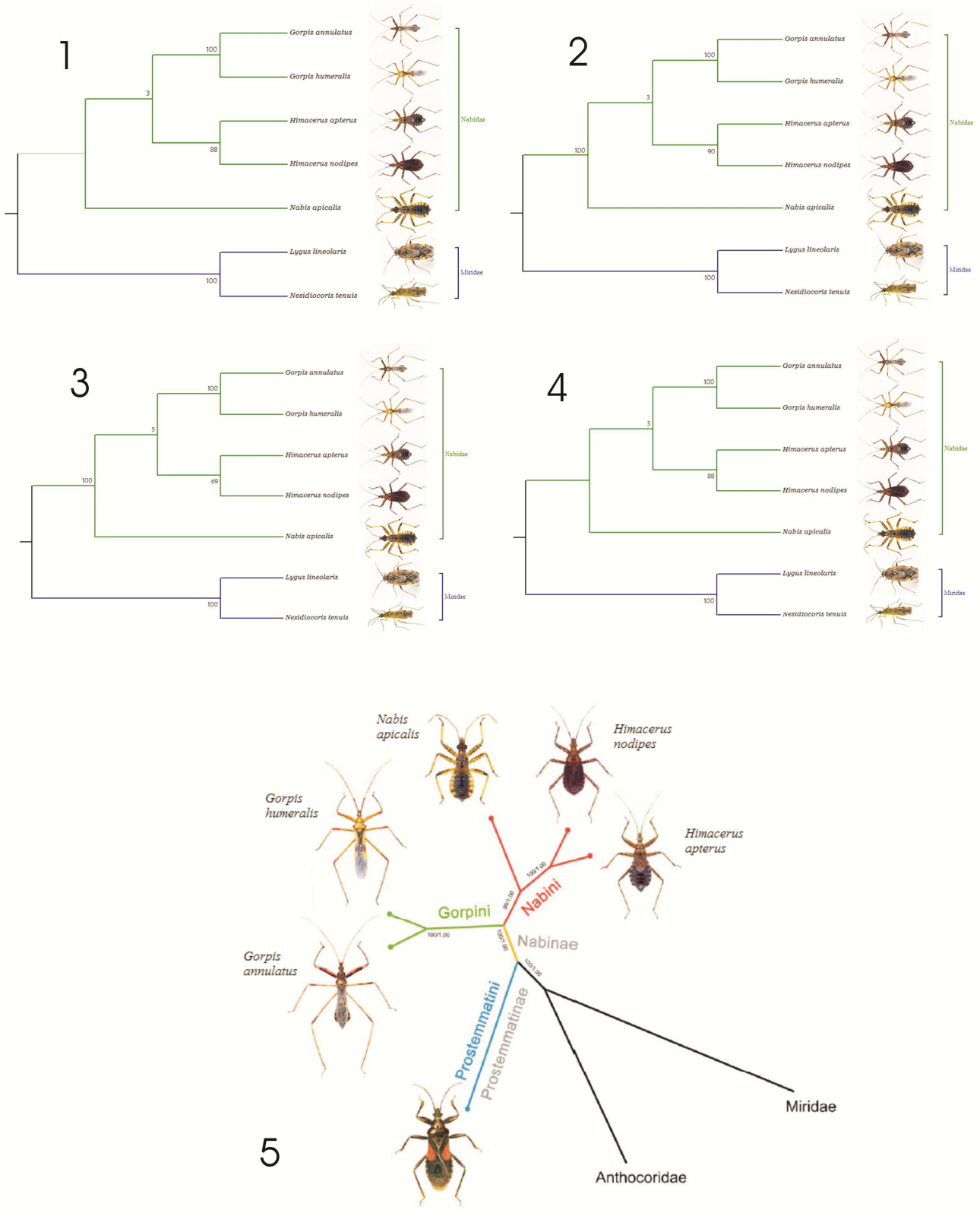
Order	Suborder	Infraorder	Family	Species	Flanking	
Hemiptera	Heteroptera	Cimicomorpha	Miridae	*Lygus lineolaris*	srRNA	$tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$
				Nesidiocoris tenuis		
			Nabidae	*Gorpis annulatus*		
				Gorpis humeralis		
				Himacerus apterus		
				Himacerus nodipes		
				Nabis apicalis		ND2
			Reduviidae	*Agriosphodrus dohrni*		$tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$
				Sirthenea flavipes		
				Triatoma dimidiata		
				Valentia hoffmanni		
		Enicocephalomorpha	Enicocephalidae	*Stenopirates* sp.	tRNAS2 (tRNAS (UCN))	lrRNA
		Gerromorpha	Gerridae	*Aquarius paludum*	srRNA	$tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$
		Leptopodomorpha	Saldidae	*Saldula arsenjevi*		
		Nepomorpha	Naucoridae	*Ilyocoris cimicoides*		
			Nepidae	*Laccotrephes robustus*		
			Notonectidae	*Enithares tibialis*		
			Pleidae	*Paraplea frontalis*		
		Pentatomomorpha	Alydidae	*Riptortus pedestris*		
			Aradidae	*Aradacanthia heissi*		$tRNA^{Gln}$-$tRNA^{Ile}$-$tRNA^{Met}$
				Neuroctenus parus		
			Berytidae	*Yemmalysus parallelus*		$tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$
			Coreidae	*Hydaropsis longirostris*		
			Largidae	*Physopelta gutta*		
			Malcidae	*Chauliops fallax*		
			Pentatomidae	*Dolycoris baccarum*		
				Nezara viridula		
			Plataspidae	*Coptosoma bifaria*		
				Megacopta cribraria		
			Pyrrhocoridae	*Dysdercus cingulatus*		
			Rhopalidae	*Stictopleurus subviridis*		
			Tessaratomidae	*Eusthenes cupreus*		
			Urostylididae	*Urochela quadrinotata*		$tRNA^{Met}$

Table 15. Comparison of the genes adjacent to the mitochondrial control region of selected species of Hemiptera: Cicadomorpha, Coleorrhyncha, Fulgoromorpha

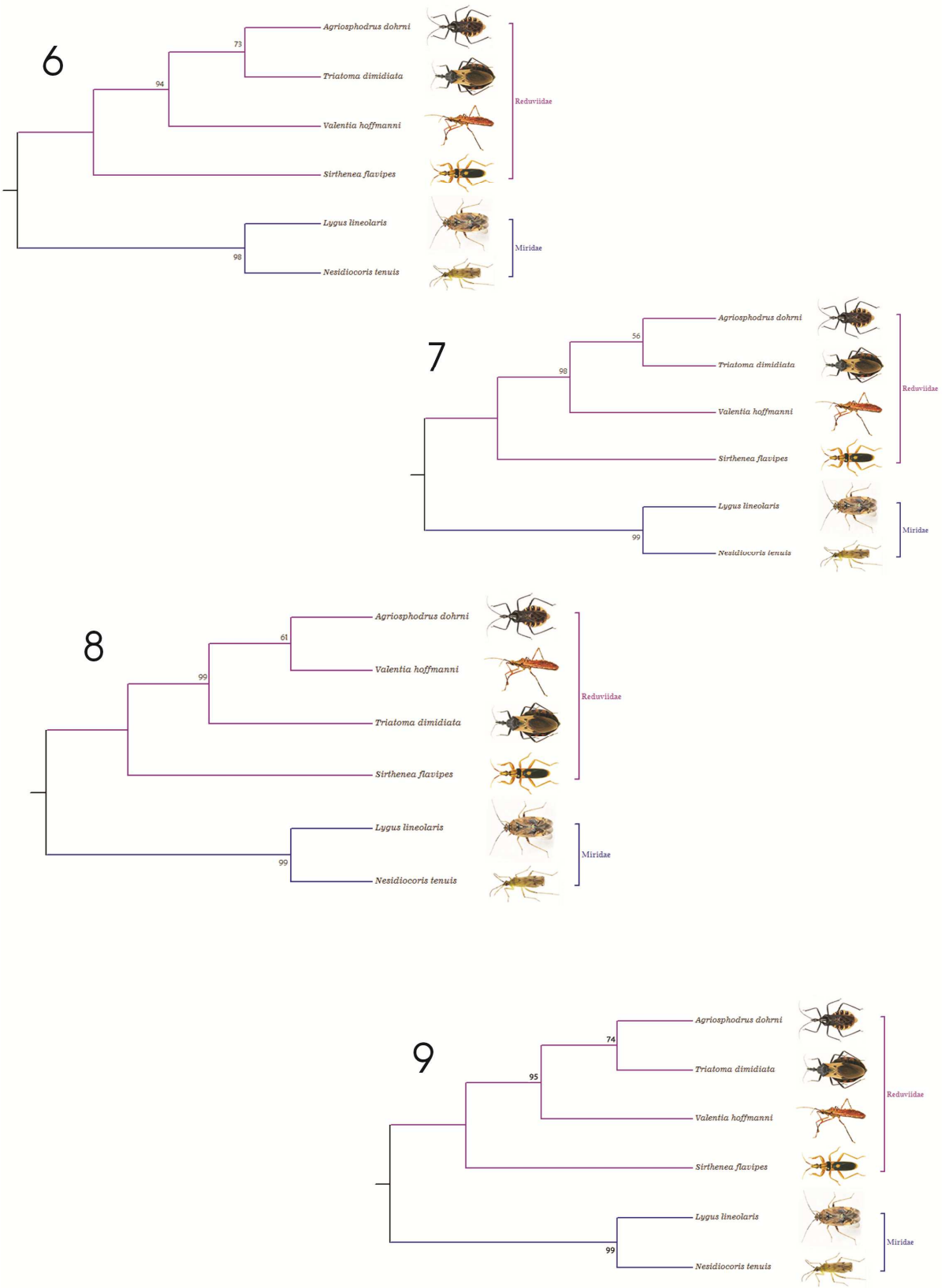
Order	Suborder	Family	Species	Flanking	
Hemiptera	Cicadomorpha	Aphrophoridae	*Philaenus spumarius*	srRNA	$tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$
		Cercopidae	*Abidama producta*		
			Callitettix versicolor		
			Paphnutius ruficeps		
	Coleorrhyncha	Peloridiidae	*Hackeriella veitchi*		
	Fulgoromorpha	Delphacidae	*Laodelphax striatella*		
			Nilaparvata lugens		
			Sogatella furcifera		
		Flatidae	*Geisha distinctissima*		
		Fulgoridae	*Laternaria candelaria*		
			Lycorma delicatula		
		Issidae	*Sivaloka damnosus*		
		Ricaniidae	*Ricania marginalis*		

Table 16. The percentage of adenine and thymine nucleotides (A+T) in mitochondrial control region of selected species of Heteroptera

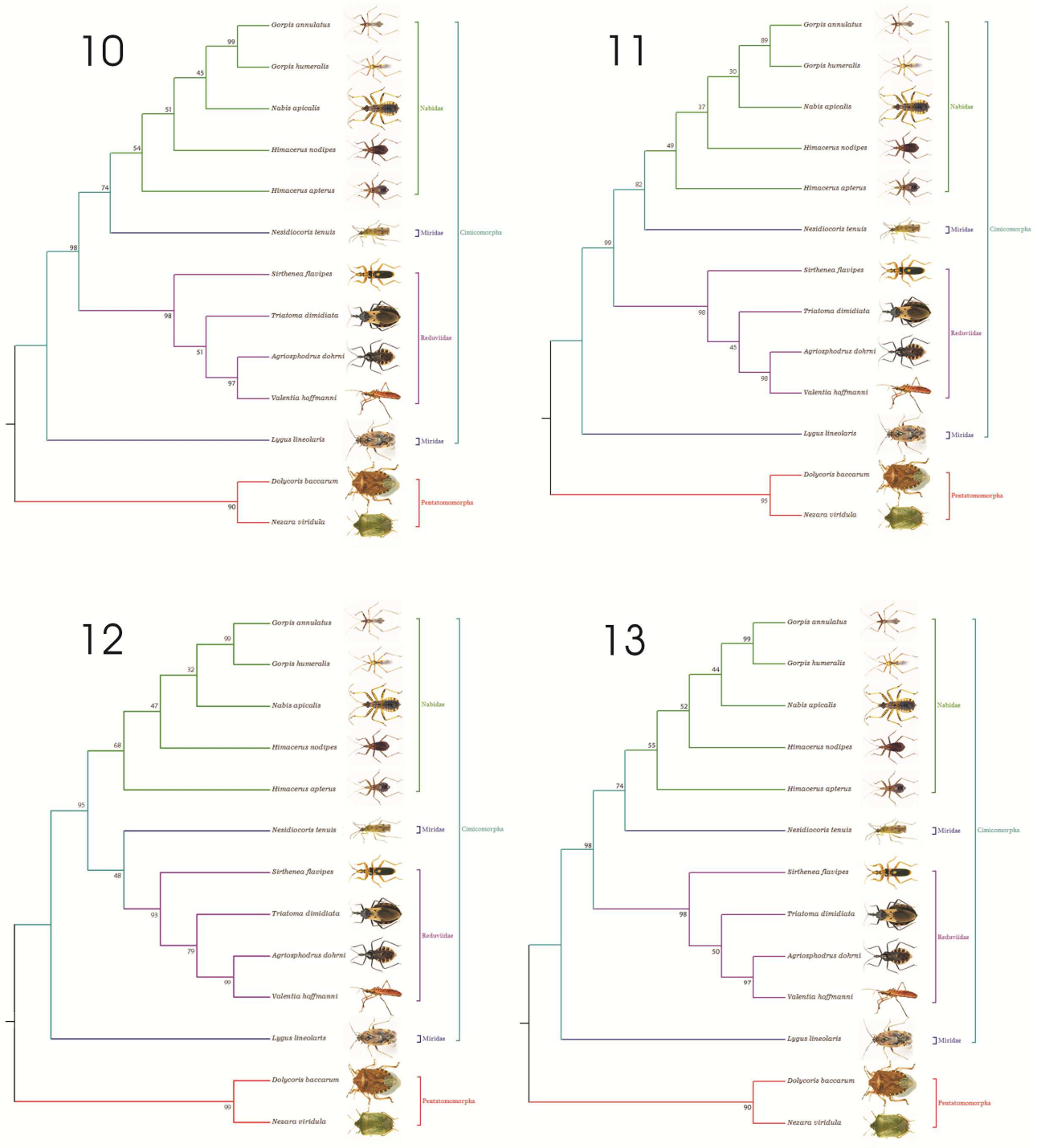
Order	Suborder	Infraorder	Family	Species	The percentage of adenine and thymine in the mitochondrial control region A+T [%]
Hemiptera	Heteroptera	Cimicomorpha	Miridae	*Nesidiocoris tenuis*	78.2
			Nabidae	*Alloeorhynchus bakeri*	75.7
			Reduviidae	*Agriosphodrus dohrni*	71.9
				Sirthenea flavipes	70.2
				Triatoma dimidiata	66.0
				Valentia hoffmanni	69.9
		Enicocephalomorpha	Enicocephalidae	*Stenopirates* sp.	74.9
		Gerromorpha	Gerridae	*Aquarius paludum*	66.2
		Leptopodomorpha	Saldidae	*Saldula arsenjevi*	77.5
		Nepomorpha	Naucoridae	*Ilyocoris cimicoides*	66.0
			Nepidae	*Laccotrephes robustus*	65.8
			Notonectidae	*Enithares tibialis*	70.4
			Pleidae	*Paraplea frontalis*	75.0
		Pentatomomorpha	Alydidae	*Riptortus pedestris*	76.4
			Aradidae	*Aradacanthia heissi*	81.5
				Neuroctenus parus	69.8
			Berytidae	*Yemmalysus parallelus*	78.9
			Coreidae	*Hydaropsis longirostris*	73.8
			Largidae	*Physopelta gutta*	75.5
			Malcidae	*Chauliops fallax*	72.4
			Pentatomidae	*Dolycoris baccarum*	74.2
				Nezara viridula	79.5
			Plataspidae	*Coptosoma bifaria*	73.8
			Pyrrhocoridae	*Dysdercus cingulatus*	79.2
			Tessaratomidae	*Eusthenes cupreus*	74.7
			Urostylididae	*Urochela quadrinotata*	79.6



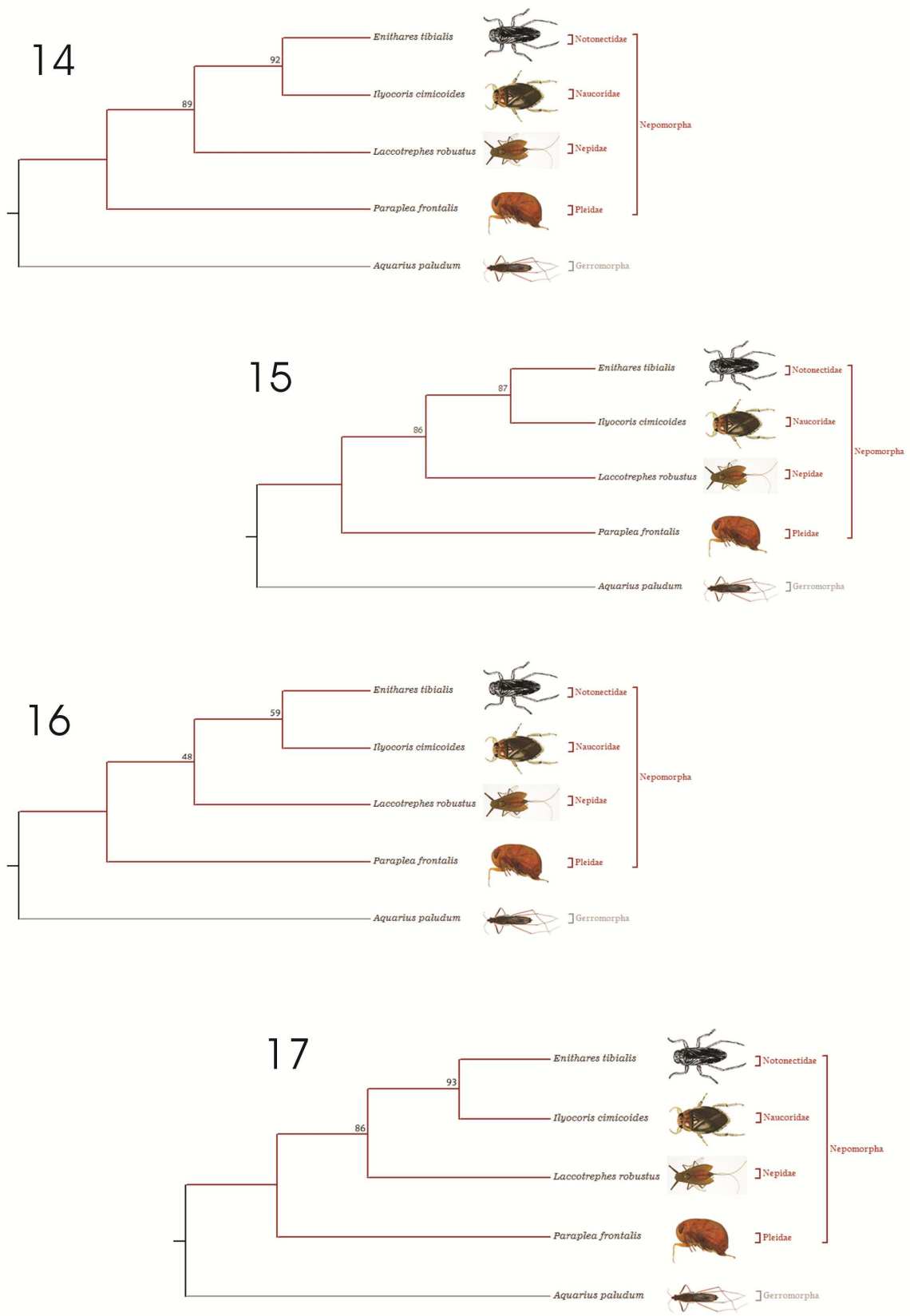
Figs. 1–4. Phylograms of selected species of the family Nabidae based on the mitochondrial control region sequences using ME (1), ML (2), MP (3), and NJ (4) method. **Fig. 5.** Maximum-Likelihood (ML) phylogram of selected species of the family Nabidae based on complete mitochondrial genome sequences (modified from Li et al. 2012b)



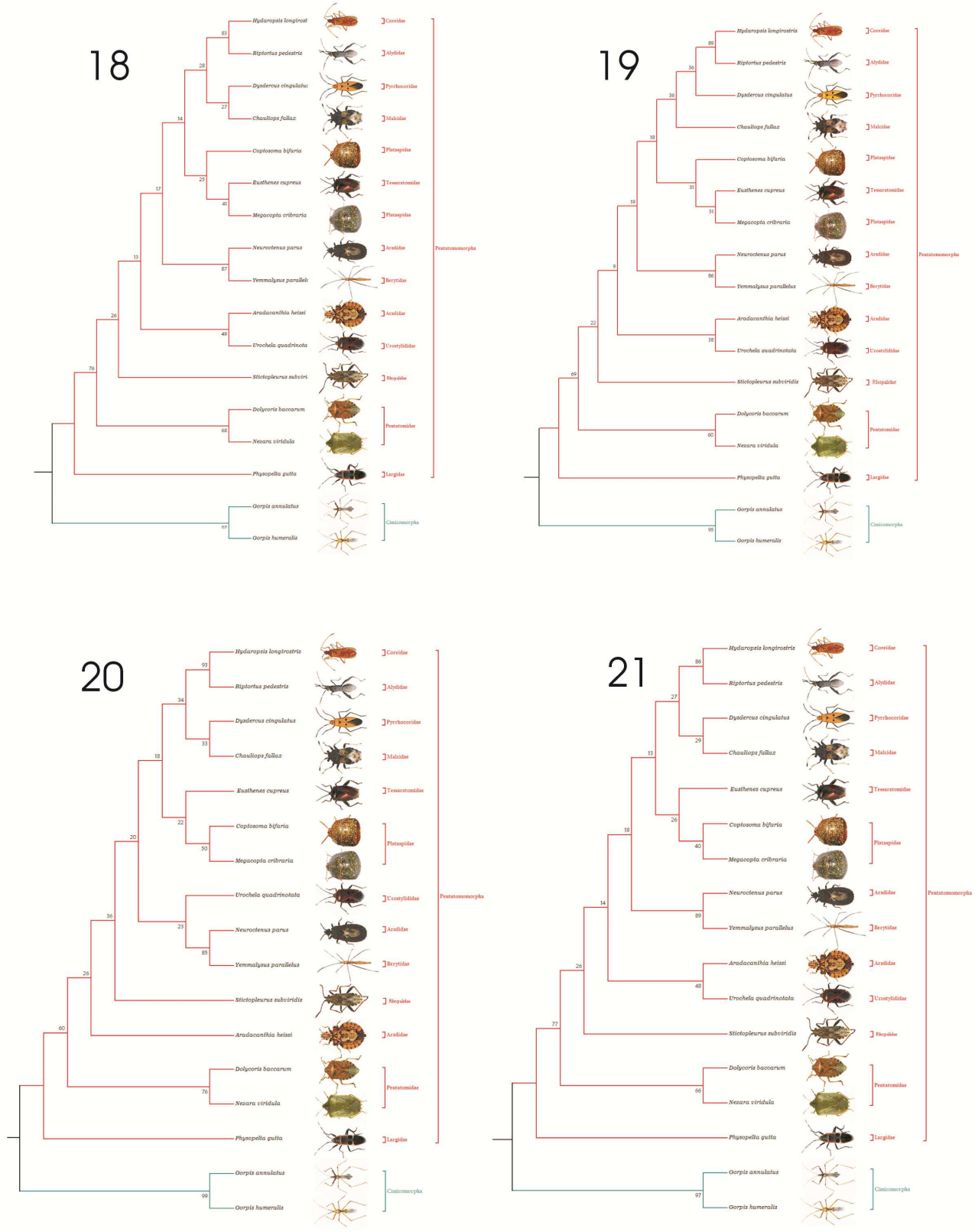
Figs. 6–9. Phylograms of selected species of the family Reduviidae based on the mitochondrial control region sequences using ME (6), ML (7), MP (8), and NJ (9) method.



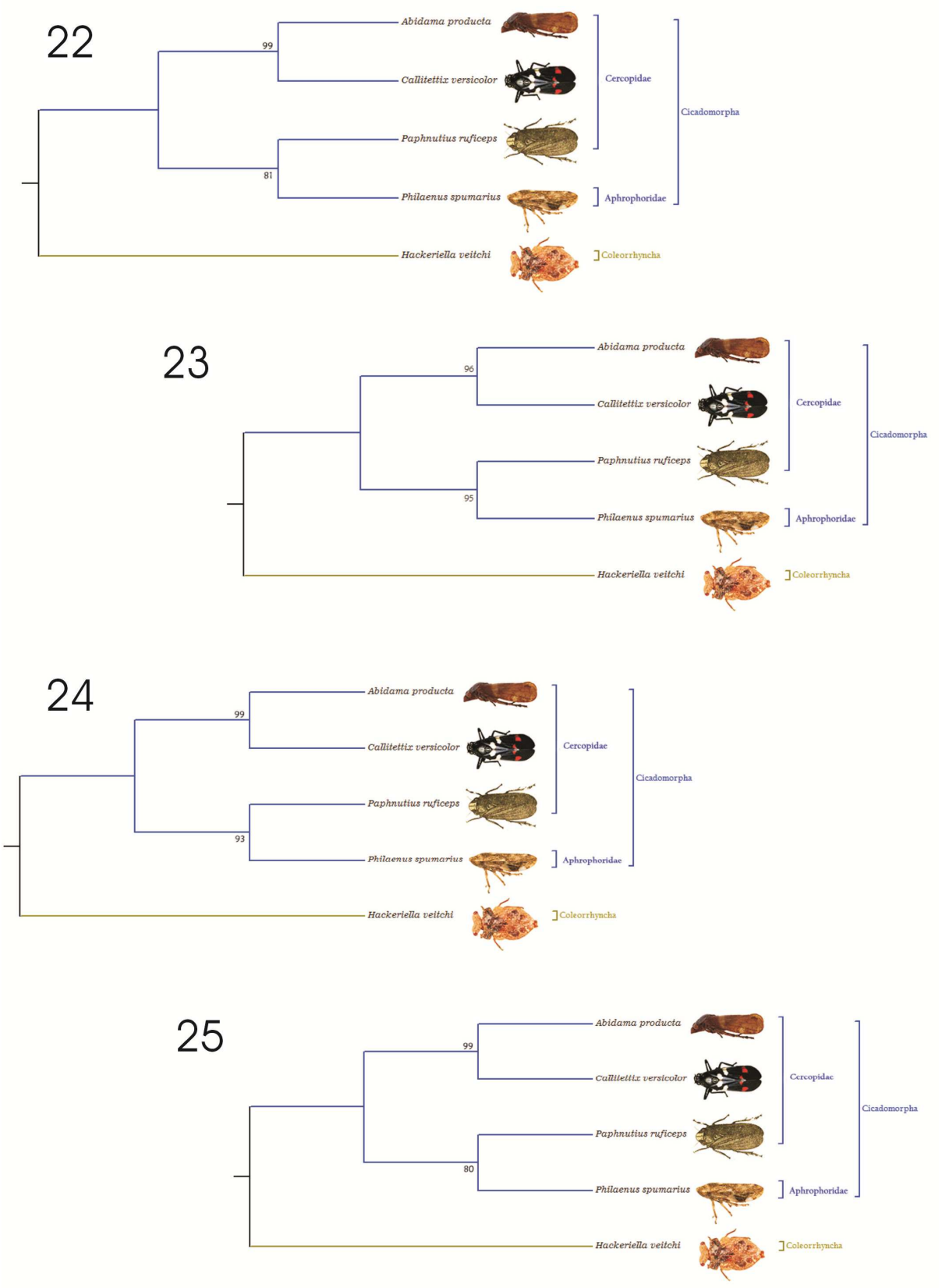
Figs. 10–13. Phylograms of selected species of the infraorder Cimicomorpha based on the mitochondrial control region sequences using ME (10), ML (11), MP (12), and NJ (13) method.



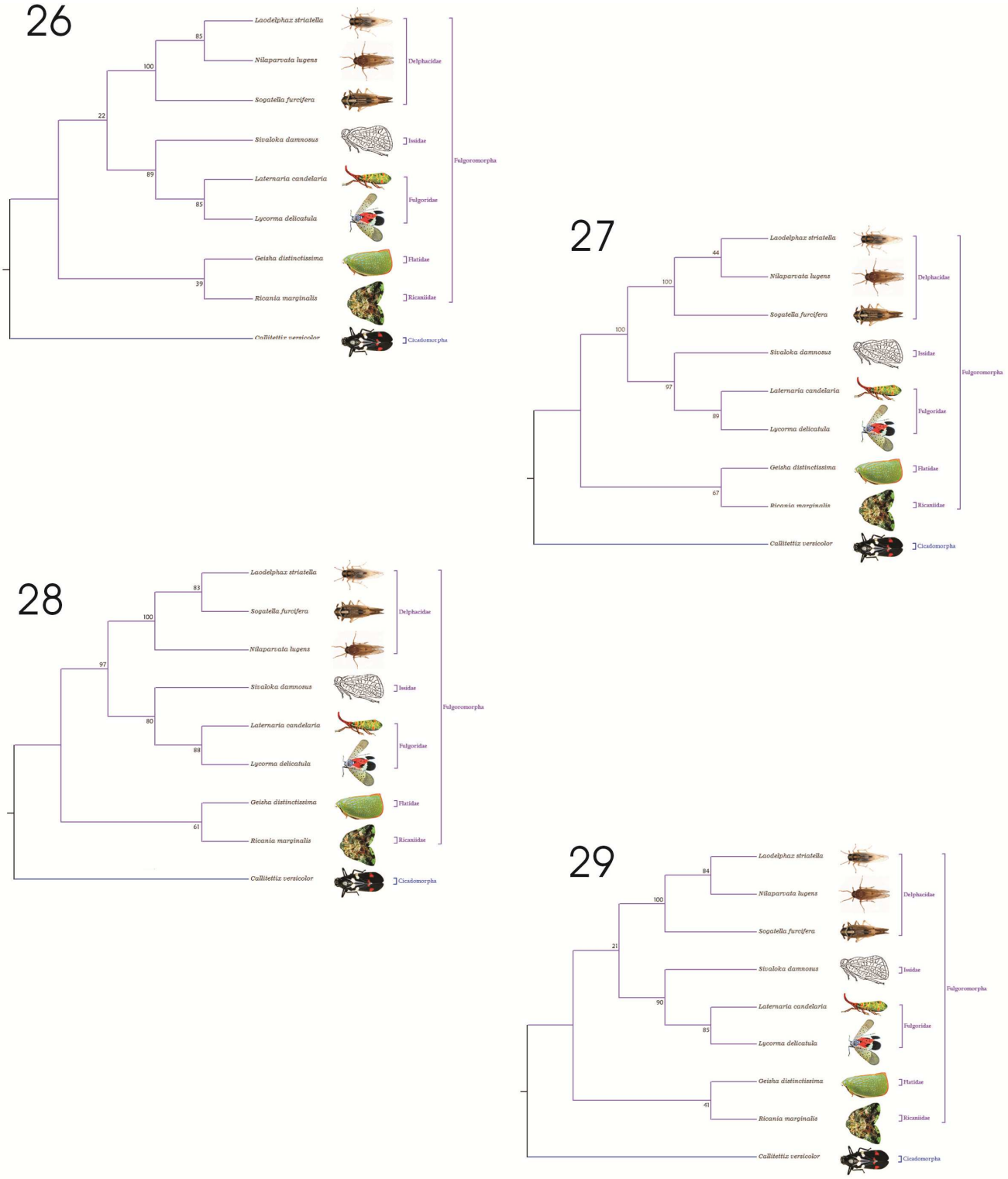
Figs. 14–17. Phylograms of selected species of the infraorder Nepomorpha based on the mitochondrial control region sequences using ME (14), ML (15), MP (16), and NJ (17) method.



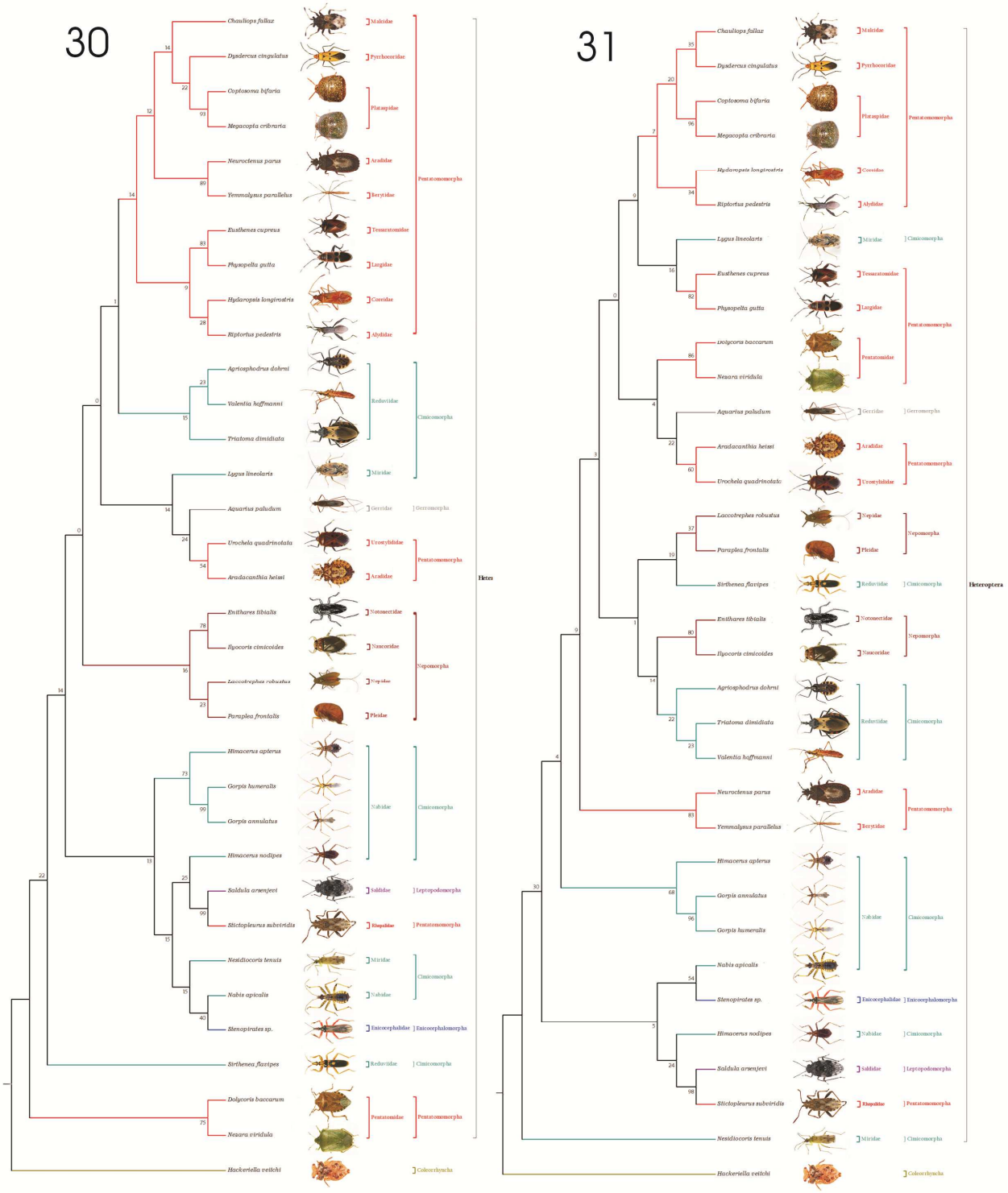
Figs. 18–21. Phylograms of selected species of the infraorder Pentatomomorpha based on the mitochondrial control region sequences using ME (18), ML (19), MP (20), and NJ (21) method.



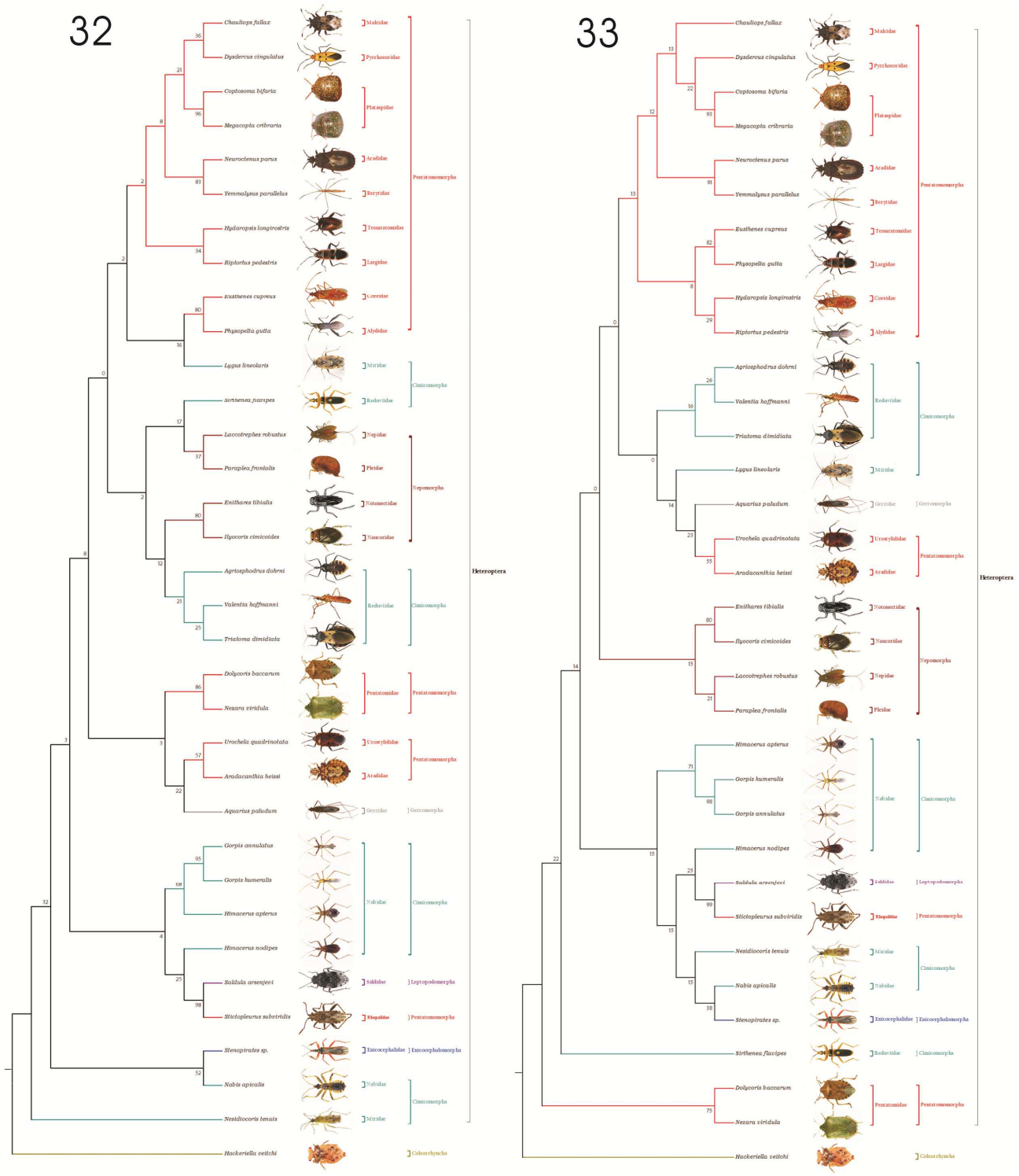
Figs. 22–25. Phylograms of selected species of the suborder Cicadomorpha based on the mitochondrial control region sequences using ME (22), ML (23), MP (24), and NJ (25) method.



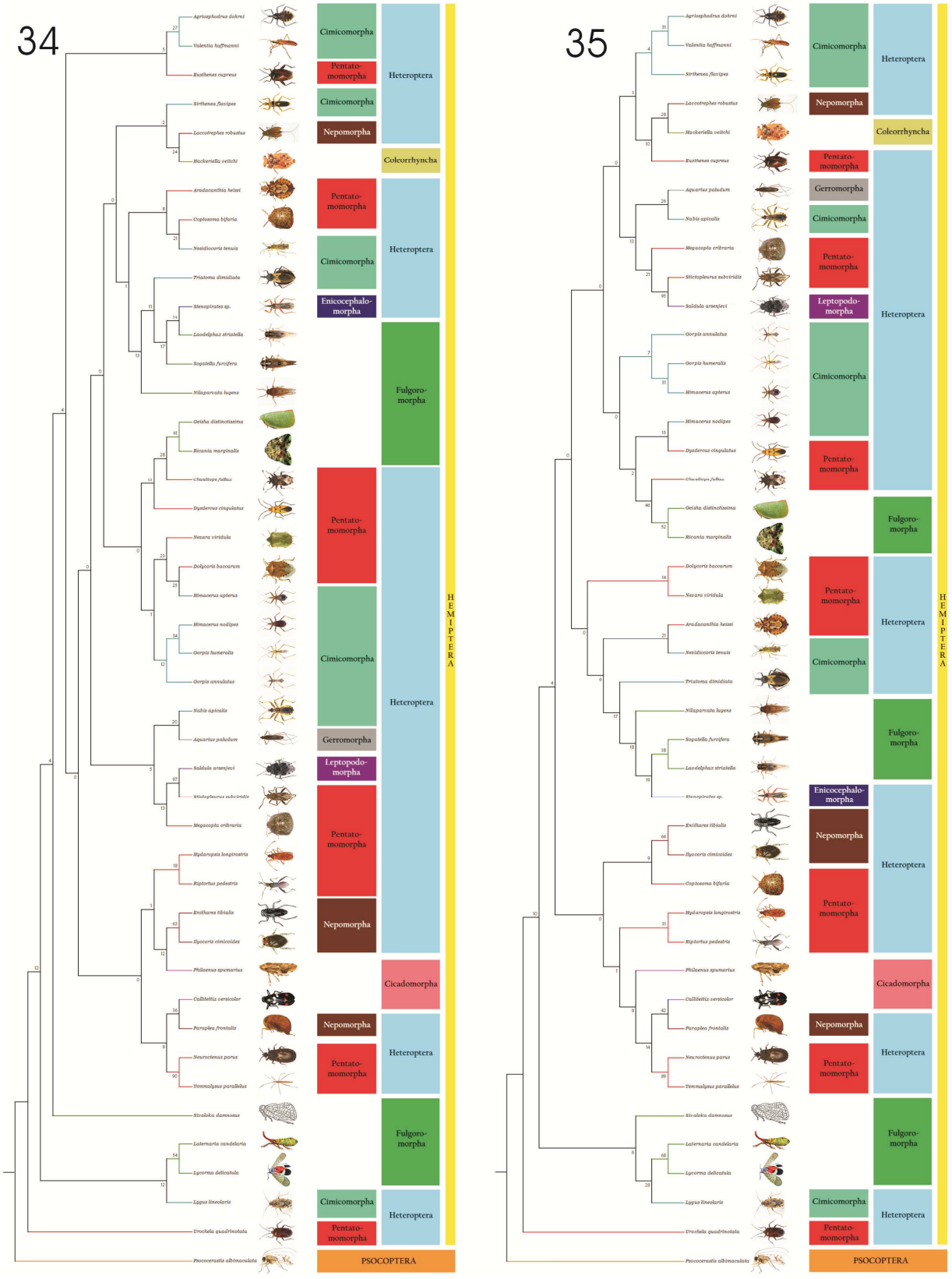
Figs. 26–29. Phylograms of selected species of the suborder Fulgoromorpha based on the mitochondrial control region sequences using ME (26), ML (27), MP (28), and NJ (29) method.



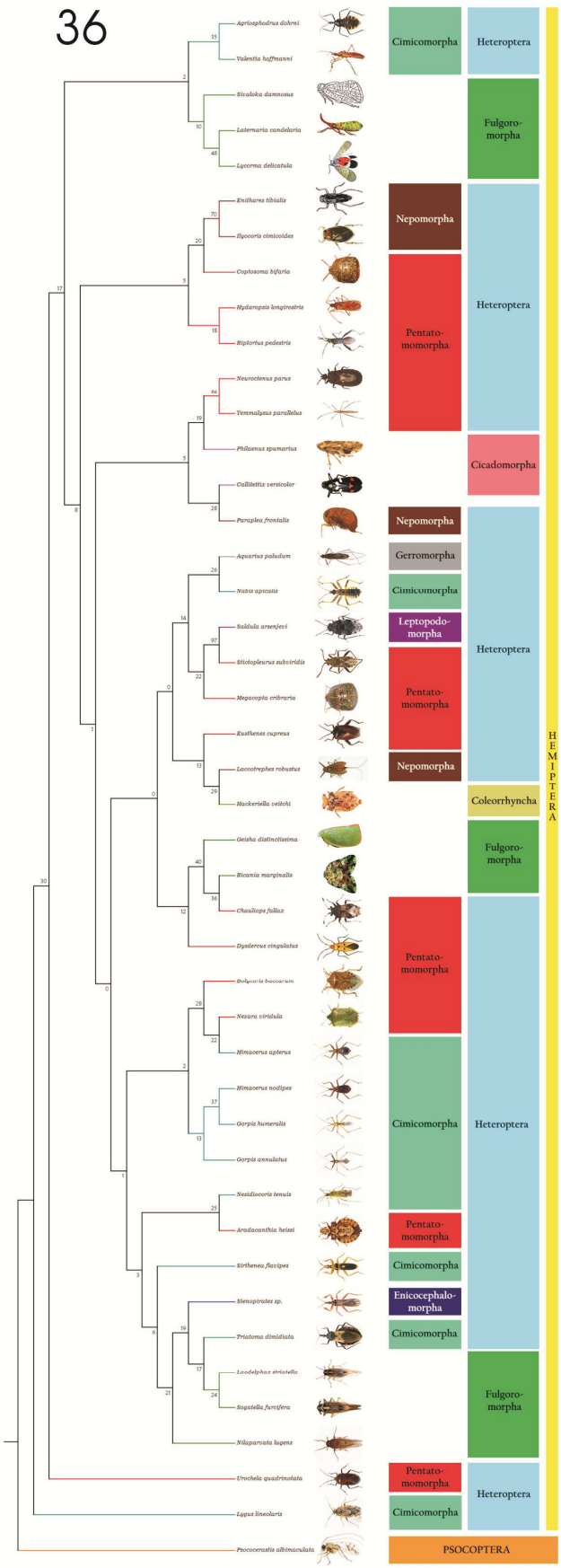
Figs. 30–31. Phylograms of selected species of the suborder Heteroptera based on the mitochondrial control region sequences using ME (30), and ML (31).



Figs. 32–33. Phylograms of selected species of the suborder Heteroptera based on the mitochondrial control region sequences using MP (32), and NJ (33) method.



Figs. 34–35. Phylograms of selected species of the order Hemiptera based on the mitochondrial control region sequences using ME (34), and ML (35).



Figs. 36–37. Phylograms of selected species of the order Hemiptera based on the mitochondrial control region sequences using MP (36), and NJ (37).

STRESZCZENIE

Ocena przydatności sekwencji regionu kontrolnego mitochondrialnego DNA do badań nad pokrewieństwem u pluskwiaków (Hemiptera)

Region kontrolny (CR) jest jedynym dużym niekodującym fragmentem w genomie mitochondrialnym pluskwiaków (Hemiptera). Podejrzewa się, iż jest on zaangażowany w procesy regulacji transkrypcji i kontroli replikacji DNA (Zhang i Hewitt 1997). Wielkość regionu kontrolnego mtDNA Hemiptera jest bardzo zróżnicowana, nawet w obrębie tego samego rodzaju. Różnice występują także w oflankowaniu CR – najczęściej są to geny srRN i $tRNA^{Ile}$-$tRNA^{Gln}$-$tRNA^{Met}$, jednak zdarzają się wyjątki (np. *Stenopirates* sp., którego CR sąsiaduje z genami tRNAS2 i lrRNA, czy *Nabis apicalis*, którego CR jest oflankowany przez srRNA i ND2). Wspólna natomiast dla wszystkich pluskwiaków jest wysoka zawartość nukleotydów adeniny i tyminy, co jest cechą charakterystyczną pod względem filogenetycznym dla owadów, dlatego inną nazwą ich regionu kontrolnego jest region bogaty w A+T. W programie MEGA 6 przeprowadzono analizy filogenetyczne z wykorzystaniem metod łączenia sąsiadów (NJ), minimalnej ewolucji (ME), maksymalnej parsymonii (MP) i największej wiarygodności (ML) w celu oceny przydatności sekwencji regionu kontrolnego mitochondrialnego DNA do badań nad pokrewieństwem u pluskwiaków. Wiele publikacji poświęcono odtwarzaniu filogenezy tej grupy organizmów z zastosowaniem różnych podjednostek genomu mitochondrialnego, jednak CR nigdy nie został poddany ocenie jako molekularny marker filogenetyczny u Hemiptera. W celu sprawdzenia przydatności sekwencji regionu kontrolnego pod kątem filogenetycznym utworzono drzewa na różnych poziomach taksonomicznych: rodziny, infrarzędu, podrzędu i rzędu. Analiza otrzymanych kladogramów prowadzi do wniosku, że region kontrolny mtDNA nie powinien być wykorzystywany jako marker molekularny w badaniach dotyczących rekonstrukcji filogenezy pluskwiaków (Hemiptera).