

Supplementary Materials: Going Deeper into High and Low Phylogenetic Relationships of Protura

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Table S1. List of species included in the phylogenetic analysis of Protura in the context of Arthropoda based on a subset of mitochondrial genes found in the same orientation. GenBank accession numbers are shown.

Crustacea	
<i>Daphnia pulex</i>	NC_000844
<i>Pagurus longicarpus</i>	NC_003058
<i>Triops cancriformis</i>	NC_004465
<i>Pollicipes polymerus</i>	NC_005936
<i>Eriocheir sinensis</i>	NC_006992
<i>Squilla empusa</i>	NC_007444
<i>Tetraclita japonica</i>	NC_008974
Hexapoda	
<i>Orthetrum triangulare</i>	AB126005
<i>Grylloblatta sculleni</i>	DQ241796
<i>Timema californicum</i>	DQ241799
<i>Sminthurus viridis</i>	EU016192
<i>Trigoniophthalmus alternatus</i>	EU016193
<i>Cryptopygus antarcticus</i>	EU016194
<i>Atelura formicaria</i>	EU084035
<i>Locusta migratoria</i>	NC_001712
<i>Tricholepidion gertschi</i>	NC_005437
<i>Periplaneta fuliginosa</i>	NC_006076
<i>Nesomachilis australica</i>	NC_006895
<i>Japyx solifugus</i>	NC_007214
<i>Tamolana tamolana</i>	NC_007702
<i>Campodea fragilis</i>	NC_008233
<i>Acerella muscorum</i>	KJ101608
<i>Acerentomon microrhinus</i>	JQ728012
<i>Sinentomon erythranum</i>	HQ199311
Myriapoda	
<i>Symphylella</i> sp	EF576853
<i>Lithobius forficatus</i>	NC_002629
<i>Narceus annularis</i>	NC_003343
<i>Thyropygus</i> sp	NC_003344
<i>Scutigera coleoptrata</i>	NC_005870
<i>Antrokoreana gracilipes</i>	NC_010221
Chelicerata	
<i>Phalangium opilio</i>	EU523757
<i>Damon diadema</i>	FJ204233
<i>Limulus polyphemus</i>	NC_003057
<i>Ornithodoros moubata</i>	NC_004357
<i>Centruroides limpidus</i>	NC_006896
<i>Achelia bituberculata</i>	NC_009724
<i>Nothopuga</i> sp	NC_009984
<i>Pseudocellus pearsei</i>	NC_009985
<i>Mastigoproctus giganteus</i>	NC_010430
<i>Calisoga longitarsis</i>	NC_010780
<i>Nymphon gracile</i>	NC_008572
<i>Ammothea carolinensis</i>	NC_014671
Outgroup	
<i>Eppiperipatus biolleyi</i>	NC_009082

Table S2. List of species included in the phylogenetic analysis of Protura based on *cox1*, *18S* and *28S* concatenated sequences. GenBank accession numbers are shown.

Species	<i>cox1</i>		<i>28S</i>		<i>18S</i>	
	Accession number	Length (bp)	Accession number	Length (bp)	Accession number	Length (bp)
<i>Eosentomon megaglenum</i>	HQ882831	657	EU557268	3670	EU557254	1945
<i>Eosentomon orientale</i>	HQ882830	657	EU557267	3710	EU557253	1946
<i>Acerentulus sinensis</i>	HQ882820	657	EU557260	3739	EU557247	1942
<i>Baculentulus tianmushanensis</i>	HQ882817	657	EF192433	3801	AY037169	1984
<i>Gracilentulus maijiawensis</i>	HQ882818	657	EF192435	3753	EU557246	1935
<i>Filientomon takanawarum</i>	HQ882822	657	EU557261	3766	EU557249	1939
<i>Acerentomon microrhinus</i>	HQ882819	657	HQ882810	3777	HQ882808	1936
<i>Acerella muscorum</i>	HQ882825	657	HQ882811	3774	HQ882809	1942
<i>Huashanentulus huashanensis</i>	HQ882821	657	EU557262	3764	EU557248	1940
<i>Nanshanentulus urumchiensis</i>	HQ882824	657	EU557264	3718	EU557250	1940
<i>Nosekiella sinensis</i>	HQ882823	657	EU557263	3684	EU557251	1942
<i>Zhongguhentomon piligeroum</i>	HQ882828	657	EU557266	3981	EU557252	1943
<i>Proturentomon chinense</i>	HQ882814	657	EU557257	3758	EU557244	1936
<i>Sinentomon erythranum</i>	HQ882826	657	EF192442	4043	AY596358	1934
<i>Huhentomon plicatunguis</i>	HQ882813	657	EU557256	3790	EU557243	1943
<i>Neocondeellum dolichotarsum</i>	HQ882815	657	EU557258	3795	AY037170	1975
<i>Hesperentomon pectigastulum</i>	HQ882812	657	EU557255	3793	EU557242	1943
<i>Eosentomon sakura</i>	HQ882829	657	EF192434	3789	AY596355	1948
<i>Lepidocampa weberi</i>	NC022675	657	EF192436	3696	AY037167	1878
<i>Occasjapyx japonicus</i>	HQ882833	657	EF192438	4255	AY596365	2040

Table S3. Species delimitation analysis (*cox1*). First column lists species as morphologically defined following BOLD or GenBank annotations. Second column reports BOLD identifiers for each specimen or GenBank accession for sequences not present in BOLD; specimens sharing the same *cox1* haplotype (after alignment trimming) are grouped using thin horizontal lines. Columns 3 to 9 show results of different analyses for species delimitation; an open cell indicate a cluster of the corresponding specimens listed in the second column; open cells carry a numerical indication of the corresponding sublineage (for cross table reference) and (where applicable) statistical support for the group expressed as posterior probabilities. Open cells with an asterisk indicate alternative clusters with similar support. Grey shading highlights morphological species that have been subdivided in two or more sublineages by at least one species delimitation method

Morphologically defined species	Haplotypes	ABGD	PTP BI	PTP ML	GMVC	BPP - 0-G(1:10); τG(1:10)	BPP - 0-G(2:100); τG(2:500)	BPP - 0-G(2:1000); τG(2:100)
<i>Acerentomon dodera</i>	PROAT026-12; PROAT030-12; PROAT031-12; PROAT034-12; PROAT036-12 to 039-12; PROAT042-12; PROAT054-12; PROAT055-12; PROAT069-12; PROAT071-12	1	1 (PP=0.95)	1 (PP=1.00)	1	1 (PP=1.00)	1 (PP=0.99)	1 (PP=1.00)
	PROAT025-12 PROAT029-12; PROAT032-12; PROAT033-12; PROAT035-12; PROAT040-12; PROAT041-12; PROAT063-12 to 066-12 PROAT027-12							
<i>Acerentomon naito</i>	PROAT001-12 PROAT016-12	1	1 (PP=1.00)	1 (PP=0.96)	1	1 (PP=1.00)	1 (PP=0.99)	1 (PP=1.00)
	PROAT005-12; PROAT019-12; PROAT024-12 PROAT020-12 PROAT006-12							
<i>Acerentomon carpaticum</i>	PROAT003-15 - PROAT007-15	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Baculentulus densus</i>	PROAT095-13	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Acerentulus tazeni</i>	PROAT051-12; PROAT056-12 to 058-12; PROAT060-12 to 062-12; PROAT072-12; PROAT076-12; PROAT079-12 to 082-12; PROAT084-12; PROAT086-12; PROAT087-12	1	1 (PP=1.00)	1 (PP=0.99)	1	1 (PP=0.98)	1 (PP=0.97)	1 (PP=1.00)
	PROAT059-12							
<i>Acerentulus exiguus</i>	PROAT028-12 PROAT070-12; PROAT049-12; PROAT075-12; PROAT077-12; PROAT083-12	1	1 (PP=1.00)	1 (PP=0.98)	1	1 (PP=0.95)	1 (PP=0.96)	1 (PP=1.00)
	PROAT045-12 PROAT048-12							
	PROAT046-12 - 047-12							
<i>Acerentomon italicum</i>	PROAT052-12	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.97)	1 (PP=0.95)	1 (PP=0.99)
	PROAT015-15 to 018-15 PROAT008-15; PROAT009-15 PROAT001-15 to 007-15							
	PROAT011-15; PROAT014-15; PROAT019-15; PROAT020-15; PROAT024-15							
	PROAT015-15 to 018-15							
<i>Essentomon sp.</i>	PROAT021-12	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Essentomon cecum</i>	PROAT073-12 PROAT068-12	1	1 (PP=1.00)	1 (PP=0.97)	1	1 (PP=1.00)	1 (PP=0.98)	1 (PP=1.00)
	PROAT078-12 PROAT085-12							
<i>Essentomon sakura</i>	HQ82829	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Essentomon megalanum</i>	HQ82831	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Essentomon orientale</i>	HQ82830	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Acerella muscorum</i>	HQ82825	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.81)	1 (PP=0.94)	1 (PP=1.00)
	PROAT023-12							
<i>Acerentulus sp. 2</i>	KJ210838-39	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Filientomon takavavaram</i>	PROAT094-13	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.96)	1 (PP=0.95)	1 (PP=1.00)
	HQ82822							
<i>Acerentulus sp. 1</i>	KJ210841; KJ210842	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Gracilentulus majavanensis</i>	HQ82818	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Yamatentomon gwui</i>	GBA11456-13	1	1 (PP=0.99)	1 (PP=0.99)	1	1 (PP=0.92)	1 (PP=0.96)	1 (PP=1.00)
	GBA11457-13							
<i>Baculentulus stannohamensis</i>	HQ82817	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Paracarella sinensis</i>	KU983763	1	1 (PP=1.00)	1 (PP=0.98)	1	1 (PP=0.85)	1 (PP=0.95)	1 (PP=1.00)
	KU983764							
	KU983757; KU983758							
	KU983759							
	KU983760							
	KU983761							
<i>Nanhanentulus urumchensis</i>	HQ82824	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Verrucosentomon lausanne</i>	KX620026	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Verrucosentomon anatoli</i>	KX620021-25	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Verrucosentomon yushanensis</i>	MK636821	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Haashanentulus haashanensis</i>	HQ82821	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Novakiella sinensis</i>	HQ82823	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Acerentomon dispar</i>	PROTA001-15; PROTA002-15 PROTA023-15 PROTA013-15	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.98)	1 (PP=0.98)	1 (PP=1.00)
	PROTA008-15; PROTA009-15 PROTA021-15; PROTA025-15							
	PROTA022-12							
	JQ728012							
<i>Acerentomon microtritus</i>	JQ728012	2	2 (PP=1.00)	2 (PP=1.00)	2	2 (PP=0.76)	2 (PP=0.94)	2 (PP=0.99)
	HQ82819							
<i>Acerentulus confinis</i>	ABPE004-17	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Acerentulus charrieri</i>	GBA988-12; GBA987-12	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Acerentulus sinensis</i>	HQ82820	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Sinentomon erythranum</i>	HQ82826	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Kenyentulus japonicus</i>	MK636822	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Anisentomon magnispinosum</i>	MK636823	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Essentomidae sp.</i>	MG319097	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.97)	1 (PP=0.95)	1 (PP=1.00)
	MG318856; MG316089							
	MG319651							
	MG320620							
<i>Anisentomon chinensis</i>	MK636824	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Zhonggushentomon piligerum</i>	HQ82828	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Essentomon communis</i>	MK636825	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Essentomon nivicolum</i>	MK636825	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Anidrentulus rapoport</i>	KJ395311; KJ395313 KJ395312	1	1 (PP=1.00)	1 (PP=0.98)	1	1 (PP=1.00)	1 (PP=0.97)	1 (PP=1.00)
<i>Huhentomon plicatunguis</i>	HQ82813	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Ionescuellum haybache</i>	PROAT043-12	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.90)	1 (PP=0.94)	1 (PP=1.00)
	PROAT008-12							
	PROAT002-12; PROAT009-12 to 011-12; PROAT013-12; PROAT014-12							
<i>Protarentomon chinense</i>	HQ82814	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Ionescuellum carpaticum</i>	PROAT004-12	1+2	1 (PP=1.00)	1 (PP=1.00)	1	* 1+2 (PP=0.50)	* 1 (PP=0.41)	1 (PP=0.71)
	PROAT007-12							
<i>Condecellum ishitani</i>	MK636827	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Neoscondecellum dolichotarsum</i>	HQ82815	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Hesperentomon bolense</i>	MF918363; MF918365-70 MF918364	1+2	1 (PP=1.00)	1 (PP=1.00)	1	1+2 (PP=0.56)	1 (PP=0.67)	1 (PP=1.00)
	KF530825 KF530824 KF530826							
<i>Hesperentomon yangi</i>	KF530825 KF530824 KF530826	1	1 (PP=0.93)	1 (PP=1.00)	1	1 (PP=0.98)	1 (PP=0.98)	1 (PP=1.00)
<i>Hesperentomon peretzgrastrum</i>	HQ82812	1	1 (PP=1.00)	1 (PP=1.00)	1	1 (PP=0.86)	1 (PP=0.94)	1 (PP=1.00)
	MF918361-62							
<i>Hesperentomon chuanghaiensis</i>	MF918360	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-
<i>Hesperentomon stringense</i>	KF530827	1	1 (PP=1.00)	1 (PP=1.00)	1	-	-	-

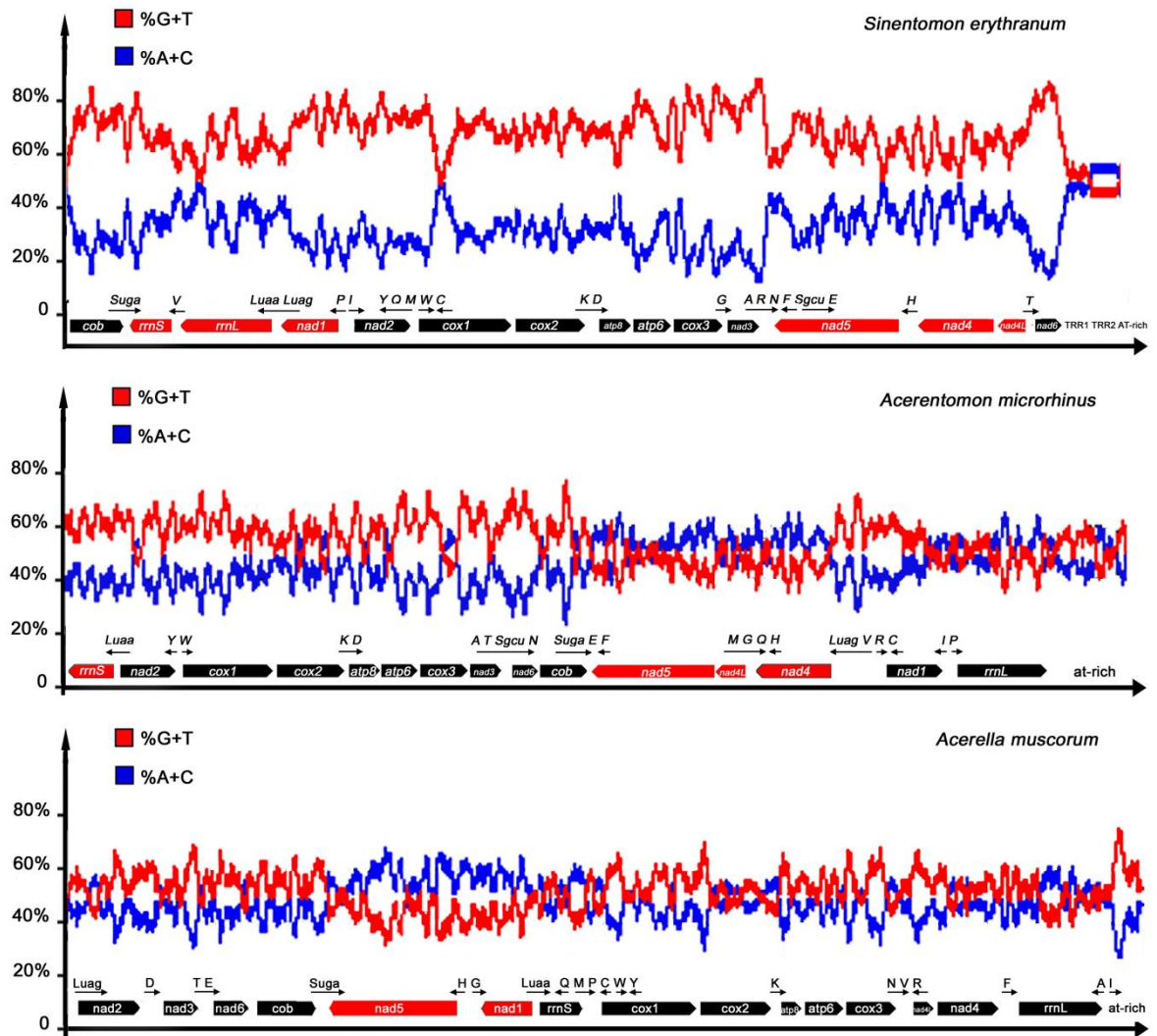


Figure S1. G+T vs. A+C content, calculated in a sliding window of 100 bp along the J-strand of the genomes of *S. erythranum*, *A. muscorum* and *A. microrhinus*. Genome maps are shown below each graph.

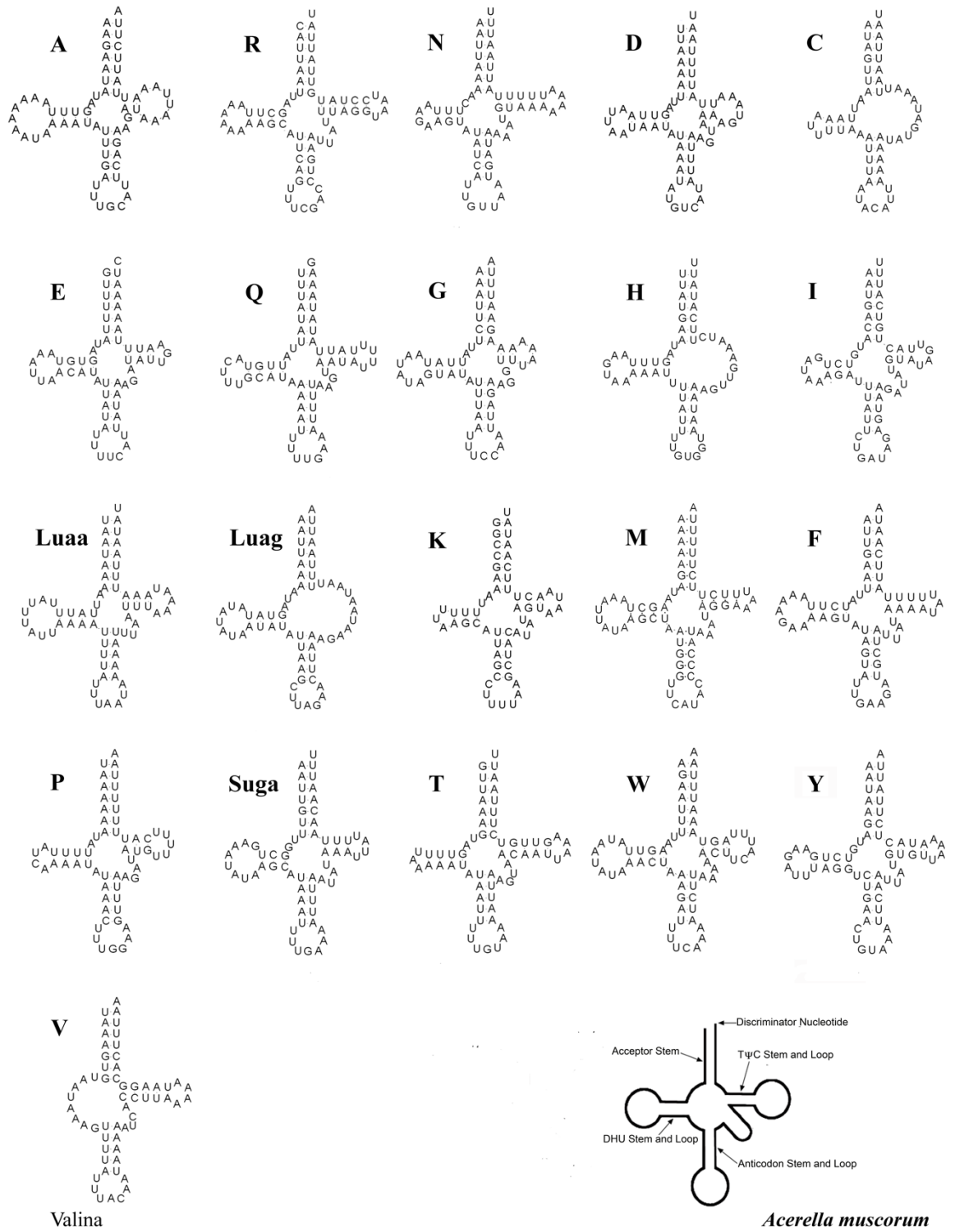


Figure S2. Secondary structure of the 21 tRNAs detected in the mitochondrial genome of *A. muscorum*. The *trnSgcu* was not identified.

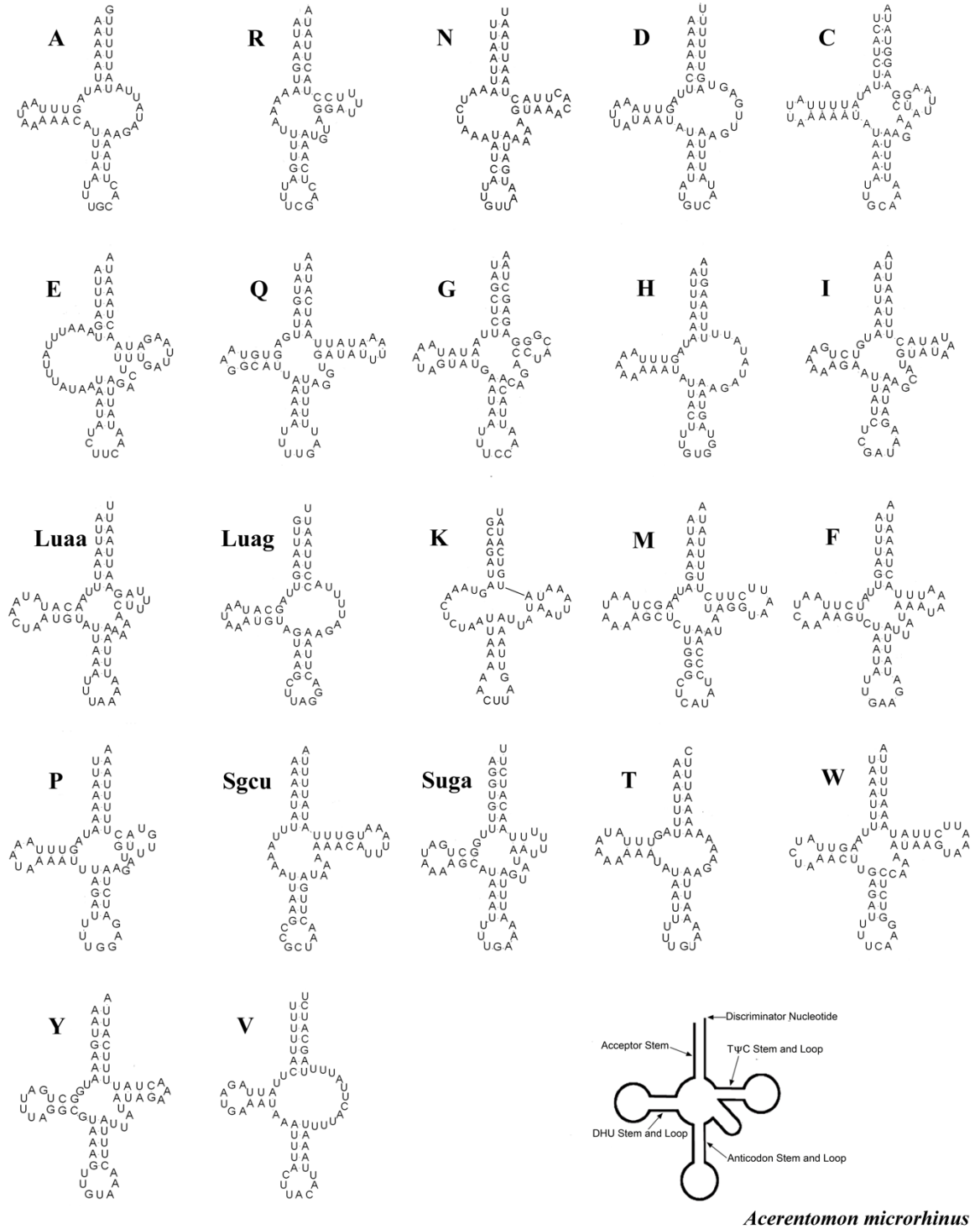
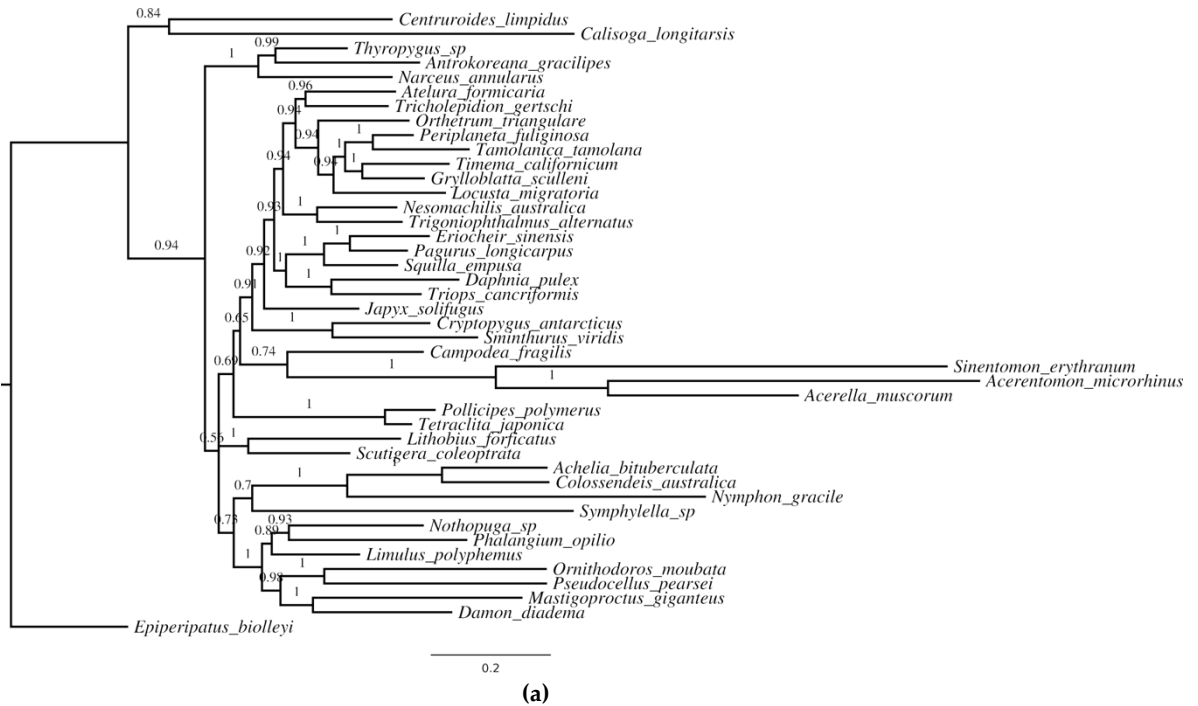


Figure S3. Secondary structure of the 22 tRNAs detected in the mitochondrial genome of *A. microrhinus*.



(a)



(b)

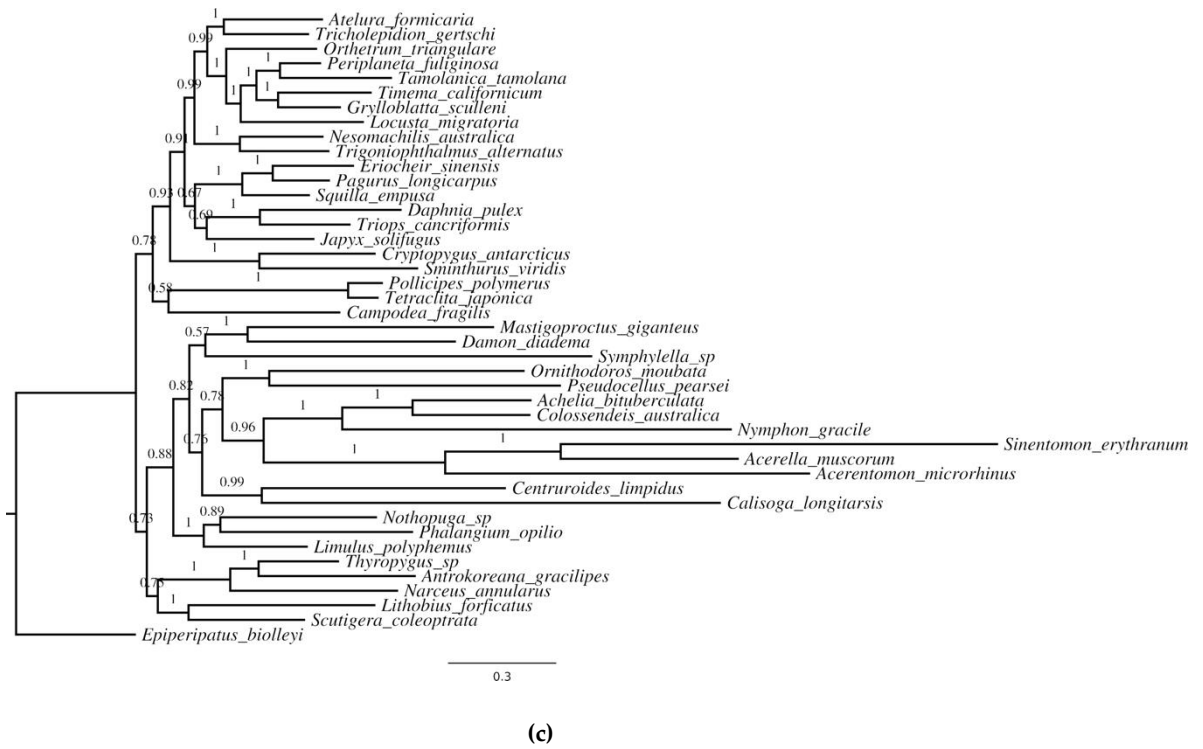


Figure S4. Phylogenetic analysis of Protura in the context of Arthropoda based on a subset of mitochondrial genes found in the same orientation. Posterior probabilities are shown above nodes. a: nucleotide dataset; b: amino acid dataset, model mixed; c: amino acid dataset, model MtPAN.

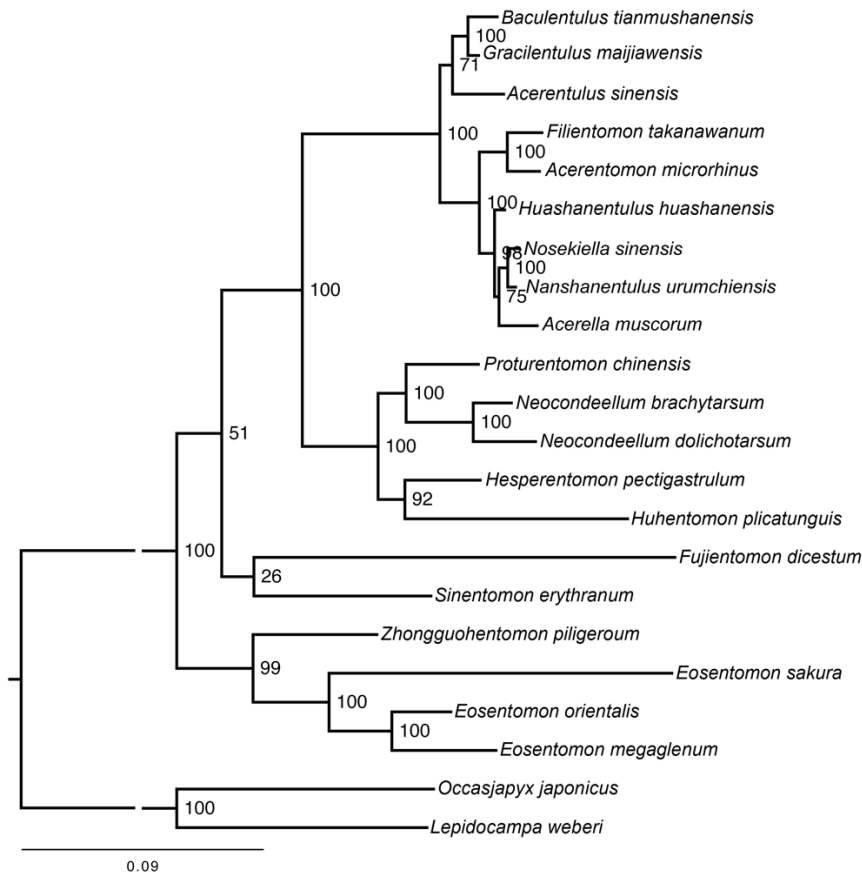


Figure S5. Phylogenetic analysis of Protura based on the reduced 18S and 28S data set. Bootstrap values shown at nodes.