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Contribution to the Molecular Phylogeny of Anthribidae (Coleoptera: Curculioniodea) Inferred from COI Sequences

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Abstract − Fungus weevils, Anthribidae, is a relatively small family, including about 3861 species worldwide, of the diverse superfamily Curculioniodea. Currently, three subfamilies are recognized within Anthribidae (Anthribinae, Choraginae and Urodontinae); however, this subfamily division is somewhat controversial and not accepted by all authors of the field. In this study, we constructed family level evolutionary histories within Anthribidae by using COI sequences and discussed the phylogenetic relations between these three subfamilies and also between tribes. Estimates of evolutionary divergence between sequences used in this study are calculated and the overall average divergence was 0,274 where minimum was 0,133 and maximum was 0,365. Phylogenetic and evolutionary histories were inferred by using 34 COI sequences representing most of the tribes and subfamilies currently recognized within Anthribidae. The phylogenetic trees were inferred by using Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods. Both method provided quite similar evolutionary histories. According to the phylogenetic trees, Anthribinae + Coraginae members together constitute a monophyletic clade, which makes currently recognized Anthribinae paraphyletic and Coraginae polyphyletic. Urodontinae seems to form a monophyletic clade with respect to used COI sequences. Tribal classification which fully seem to depends on morphological characters are useful for practical taxonomic purposes but most tribes (those represented with more than one species/sequence) does not constitute monophyletic clades.

Keywords − *Anthribidae, COI barcode, DNA barcode, fungus weevils, molecular phylogeny*

1. Introduction

Fungus weevils Anthribidae Billberg, 1820, constitute a family belonging to diverse superfamily Curculioniodea (Coleoptera) Latreille, 1802, of which most members are associated with the fungus and decaying woods [1,2]. Currently, the family Anthribidae include about 3861 species spread over worldwide [3,4]. Although it is somewhat controversial, three subfamilies are recognized within Anthribidae: Anthribinae Billberg, 1820 (28 tribes, 308 genera, 3148 species), Choraginae Kirby, 1819 (five tribes, 62 genera, 630 species) and Urodontinae Thomson, 1859 (eight genera, 83 species) [4].

Cytochrome c oxidase I (COI or COXI) gene sequences are extensively used as DNA barcodes for molecular species identification and in phylogenetic studies [5,6]. Thousands of COI barcodes belonging to all living forms are now deposited publicly in several databases including NCBI (GenBank), BOLD etc. and those barcode sequences are used by researchers freely. In GenBank nucleotide database, there are 1383 sequences belonging to species of Anthribidae, and 422 of them are COI sequences i.e., DNA barcodes (https://www.ncbi.nlm.nih.gov/nuccore/?term=Anthribidae+COI).

In recent revisional and phylogenetic research, superfamily Curculionoidea includes seven families of which

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Anthribidae is positioned as a basal clade [4,7]. As stated above, three subfamilies (Anthribinae, Coraginae and Urodontinae) are recognized in Anthribidae; however, there are still discussions about subfamily separations. Although Urodontinae is treated as a separate subfamily by Crowson [8] and Rheinheimer [3], Mermudes and Leschen [4] considered Urodontinae as a part of Anthribinae. These subfamilies are mainly distinguished by morphological features and this group lacks sufficient molecular phylogenetic research. Anthribinae and Choraginae are distinguished by several morphological traits; the shape of the first two antennal segments, the position of the antennal scrobe, and the shape of the female genitalia (Holloway 1982).

The main goal of this paper is to contribute current understanding of the family level phylogenetic relationships within Anthribidae, and to discuss the subfamilial and tribal classification.

2. Materials and Methods

The first papers dealing with family level classification of weevils (Curculioniodea) date back to the 19th century [10-13]. Since then, 11 to 22 families were recognized [14]. Later with the application of phylogenetic methods, the number of major lineages was reduced to 6 families [7,15,16].

The materials of this study were COI sequences belonging to 34 species/specimens, 27 genera, 14 tribes and three subfamilies currently recognized in Anthribidae. Two sequences belonging to *Cimberis attelaboides* (Fabricius, 1787) (Nemonychidae) and *Curculio glandium* Marsham, 1802 (Curculionidae) were used as outgroup taxa. All COI sequences were provided from GenBank nucleotide database and given in Table 1.

Molecular Evolutionary Genetic Analysis (MEGA-X) software was used for all analysis [17]. COI Sequences taken from GenBank nucleotide database were assembled in MEGA-X to generate alignments. Multiple Sequence Comparison by Log-Expectation (MUSCLE) [18] program was used to generate multiple alignments of COI sequences. The evolutionary distances between sequences were measured by using the Kimura 2-parameter model [19]. The evolutionary history was inferred by using Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods, and phylogenetic trees are given separately.

3. Results and Discussion

In this paper, we used 34 COI sequences representing most of the tribes and genera of Anthribidae and two sequences as outgroup taxa. Estimates of evolutionary divergence between sequences are given in Table 2. The overall average divergence was 0,274 where minimum was 0,133 and maximum was 0,365.

Table 1. Taxa used in the phylogenetic analysis

Subfamily	Tribus	Genus	Species	GenBank Acc. No.
Anthribinae	Anthribini	*Anthribus*	*Anthribus fasciatus* Forster, 1770	KM285875
			Anthribus nebulosus Forster, 1770	MG055246
	Corrhecerini	*Disphaerona*	*Disphaerona chinensis* Frieser, 1995	HQ987085
	Cratoparini	*Euparius*	*Euparius marmoreus* (Olivier, 1800)	HM433176
	Discotenini	*Acanthopygus*	*Acanthopygus griseus* Montrouzier, 1860	KX683369
		Pseudeuparius	*Pseudeuparius sepicola* (Fabricius, 1792)	KU908682
		Eucorynus	*Eucorynus crassicornis* (Fabricius, 1802)	KX053198

Table 1. (Continued)

Subfamily	**Tribus**	**Genus**	**Species**	**GenBank Acc. No.**
Anthribinae	Jordanthribini	*Jordanthribus*	*Jordanthribus* sp.	KX053540
		Dinema	*Dinema filicornis* Fairmaire, 1849	KX052591
	Platyrhinini	*Platyrhinus*	*Platyrhinus resinosus* (Scopoli, 1763)	KU917082
	Platystomini	*Phloeobius*	*Phloeobius gigas* (Fabricius, 1775)	HM431488
		Platystomos	*Platystomos albinus* (Linnaeus, 1758)	KU916837
		Toxonotus	*Toxonotus cornutus* (Say, 1831)	HM433055
	Stenocerini	*Allandrus*	*Allandrus therondi* (Tempère, 1954)	KM286294
			Allandrus undulates (Panzer, 1795)	MZ607386
	Trigonorhinini	*Trigonorhinus*	*Trigonorhinus alternatus* (Say, 1826)	MF640977
			Trigonorhimus tomentosus (Say, 1826)	FJ867819
	Tropiderini	*Gonotropis*	*Gonotropis dorsalis* (Thunberg, 1796)	MZ610748
		Tropideres	*Tropideres albirostris* (Schaller, 1783)	KM452571
	Zygaenodini	*Dissoleucas*	*Dissoleucas niveirostris* (Fabricius, 1798)	MZ606968
		Noxius	*Noxius curtirostris* (Mulsant, 1861)	MN183027
		Ormiscus	*Ormiscus walshii* (LeConte, 1876)	KR485751
		Rhaphitropis	*Rhaphitropis marchica* (Herbst, 1797)	HQ954422
			Rhaphitropis oxyacanthae (Brisout, 1863)	KM285872
		Xynotropis	*Xynotropis* sp.	KM006611
Choraginae	Araecerini	*Araecerus*	*Araecerus fasciculatus* (DeGeer, 1775)	KM446808
	Choragini	*Choragus*	*Choragus sheppardi* Kirby, 1819	KM444886
		Melanopsacus	*Melanopsacus grenieri* (Barneville, 1867)	MN182766
		Styphlochoragus	*Styphlochoragus trachodes* Frieser, 1978	KJ672228
Urodontinae	Urodontini	*Bruchela*	*Bruchela conformis* (Gyllenhal, 1833)	KM448962
			Bruchela rufipes (Olivier, 1790)	MK892264
			Bruchela schusteri (Schilsky, 1912)	MK892233
			Bruchela suturalis (Fabricius, 1792)	MK892262
		Urodontus	*Urodontus mesemoides* Louw, 1993	FJ867818
Outgroup taxa		Nemonychidae	*Cimberis attelaboides* (Fabricius, 1787)	MZ658256
		Curculionidae	*Curculio glandium* Marsham, 1802	KC784023

Table 2. Estimates of evolutionary divergence between sequences. The numbers of base substitutions per site from between sequences are shown. Analyses were conducted using the Kimura 2-parameter model

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
1 *Anthribus fasciatus*																																	
2 *Anthribus neb ulosus*	0,23																																
3 *Disphaerona chinensis*	0,28	0,27																															
4 *Euparius mamorews*	0,26	0,31	0,25																														
5 *Acanthopygus griseus*	0,25	0,24	0,27	0,27																													
6 *Pseudeuparius spicola*	0,29	0,31	0,28	0,28	0,29																												
7 *Enconyus crassico mils*	0,27	0,28	0,33	0,29	0,29	0,33																											
8 *Jordanthribus sp.*	0,25	0,27	0,26	0,25	0,28	0,25	0,29																										
9 *Dinema filicomis*	0,24	0,27	0,28	0,25	0,30	0,30	0,25	0,22																									
10 *Platyminus resinosus*	0,23	0,23	0,24	0,24	0,25	0,25	0,26	0,27	0,22																								
11 *Phloeobitus gigas*	0.27	0,26	0,29	0,32	0,31	0,32	0,33	0,28	0,25	0,28																							
12 *Platysto mos albinus*	0,27	0,29	0,24	0,24	0,28	0,27	0,33	0,29	0,27	0,24	0,25																						
13 *Toxonotus comutus*	0,29	0,33	0,22	0,25	0,34	0,30	0,31	0,29	0,28	0,29	0,28	0,25																					
14 *Allandrus therondi*	0,29	0,26	0,29	0,27	0,29	0,29	0,30	0,24	0,24	0,28	0,26	0,30	0,32																				
15 *Allandrus undulatus*	0.26	0,28	0,30	0,29	0,27	0,30	0,30	0,28	0,24	0,24	0,29	0,30	0,31	0,13																			
16 *Trigono rhinus altematus*	0,20	0,20	0,26	0,26	0,26	0,28	0,27	0,28	0,20	0,19	0,26	0,26	0,26	0,23	0,21																		
17 *Trigono rhinus tomentosus*	0,21	0,20	0,25	0,29	0,28	0,28	0,32	0,23	0,24	0,20	0,27	0,26	0,32	0,23	0,26	0,19																	
18 *Gomotropis dorsalis*	0,28	0,31	0,31	0,28	0,30	0,29	0,27	0,28	0,26	0,28	0,29	0,22	0,27	0,28	0,27	0,27	0,28																
19 *Tro pideres albirostris*	0,26	0,31	0,28	0,27	0,29	0,28	0,31	0,28	0,27	0,28	0,29	0,26	0,28	0,26	0,31	0,26	0,26	0,21															
20 *Dissoleucas niveiro stris*	0,27	0,23	0,26	0,28	0,28	0,26	0,30	0,26	0,27	0,24	0,29	0,30	0,31	0,25	0,25	0,26	0,22	0,28	0,25														
21 *Noxius curtirostris*	0,25	0,22	0,25	0,25	0,23	0,26	0,23	0,20	0,20	0,20	0,25	0,21	0,27	0,24	0,27	0,22	0,25	0,28	0,25	0,24													
22 *Ormiscus ralshii*	0,25	0,27	0,28	0,29	0,25	0,25	0,29	0,24	0,30	0,26	0,28	0,25	0,31	0,30	0,29	0,28	0,27	0,26	0,29	0,28	0,23												
23 *Rhaphitropis marchica*	0,25	0,27	0,29	0,28	0,28	0,23	0,31	0,21	0,23	0,26	0,28	0,25	0,29	0,24	0,24	0,25	0,26	0,23	0,26	0,28	0,23	0,28											
24 *Rhaphitropis oxpacanthae*	0,22	0,20	0,26	0,27	0,24	0,25	0,32	0,28	0,28	0,24	0,27	0,32	0,30	0,25	0,24	0,22	0,22	0,31	0,28	0,23	0,24	0,29	0,26										
25 *Xymotropis sp.*	0,24	0,28	0,26	0,25	0,27	0,25	0,28	0,24	0,26	0,24	0,30	0,22	0,30	0,31	0,31	0,28	0,27	0,26	0,26	0,25	0,21	0,25	0,27	0,24									
26 *Araecents fasciculatus*	0,34	0,32	0,32	0,32	0,31	0,36	0,33	0,30	0,30	0,32	0,31	0,29	0,32	0,32	0,33	0,33	0,34	0,32	0,36	0,36	0,29	0,34	0,32	0,32	0,33								
27 *Choragus sheppardi*	0,21	0,25	0,25	0,27	0,23	0,28	0,27	0,25	0,24	0,21	0,26	0,28	0,30	0,30	0,27	0,22	0,27	0,31	0,29	0,28	0,22	0,28	0,25	0,23	0,27	0,34							
28 *Melanepsacus grenien*	0,22	0,22	0,24	0,29	0,20	0,26	0,24	0,23	0,22	0,19	0,26	0,26	0,29	0,25	0,28	0,22	0,20	0,25	0,28	0,27	0,17	0,24	0,24	0,22	0,22	0,28	0,21						
29 *Styphlochoragus trachodes*	0,26	0,25	0,29	0,29	0,21	0,29	0,31	0,23	0,26	0,22	0,29	0,27	0,34	0,29	0,31	0,29	0,28	0,28	0,31	0,26	0,22	0,24	0,25	0,26	0,21	0,30	0,25	0,20					
30 *Bruchela conformis*	0,33	0,30	0,30	0,35	0,28	0,24	0,33	0,29	0,31	0,29	0,32	0,30	0,34	0,31	0,31	0,33	0,28	0,30	0,32	0,29	0,27	0,33	0,25	0,26	0,28	0,33	0,28	0,25	0,25				
31 *Bruchela rufipes*	0,28	0,28	0,29	0,33	0,29	0,27	0,36	0,29	0,29	0,30	0,34	0,30	0,35	0,30	0,34	0,32	0,31	0,31	0,34	0,30	0,27	0,33	0,28	0,30	0,26	0,31	0,29	0,24	0,27	0,16			
32 *Bruchela sch usteri*	0,29	0,25	0,30	0,35	0,21	0,28	0,32	0,27	0,29	0,23	0,31	0,29	0,34	0,30	0,28	0,26	0,25	0,32	0,32	0,27	0,26	0,26	0,27	0,26	0,27	0,36	0,23	0,22	0,24	0,18	0,19		
33 *Bruchela suturalis*	0,30	0,30	0,29	0,34	0,26	0,26	0,35	0,26	0,28	0,26	0,30	0,33	0,34	0,27	0,28	0,28	0,26	0,33	0,34	0,29	0,28	0,28	0,25	0,25	0,30	0,35	0,26	0,25	0,29	0,16	0,19	0,15	
34 *Urodontus mesemoides*	0,29	0,30	0,33	0,31	0,24	0,31	0,32	0,29	0,27	0,25	0,30	0,30	0,35	0,26	0,25	0,25	0,24	0,28	0,30	0,28	0,26	0,31	0,30	0,26	0,29	0,35	0,23	0,22	0,27	0,26	0,26	0,20	0,23

We constructed evolutionary scenarios by using Neighbor-joining and maximum likelihood methods. The phylogenetic scenarios inferred from NJ and ML analyses are given in Figures 1 and 2, respectively. The topologies of both trees were similar to each other with slight differences. According to evolutionary history inferred from NJ method (Figure 1), Anthribinae+Coraginae constitute a monophyletic clade where currently recognized Anthribinae was paraphyletic with respect to Choraginae members placed within Antribinae clades. Urodontinae seems to be monophyletic (a clade descended from a common ancestor or ancestral group) with respect to sequences used in this study. As to tribal classification, some tribes (i.e., Tropiderini and Urodontini) seems to be monophyletic, most of the tribes were paraphyletic (a clade descended from a common ancestor or ancestral group, but not including all the descendant groups) or polyphyletic (a clade derived from more than one ancestor). This shows that tribal classification doesn't reflect evolutionary history but is used for practical reasons to identify specimens. Evolutionary history inferred from ML method (Figure 2) was quite similar to NJ scenario with respect to subfamily placements. The only major difference was the placement of Coraginae members within Anthribinae clades. Tribal classification also seems mostly not monophyletic with the same few monophyletic tribes as in NJ scenario.

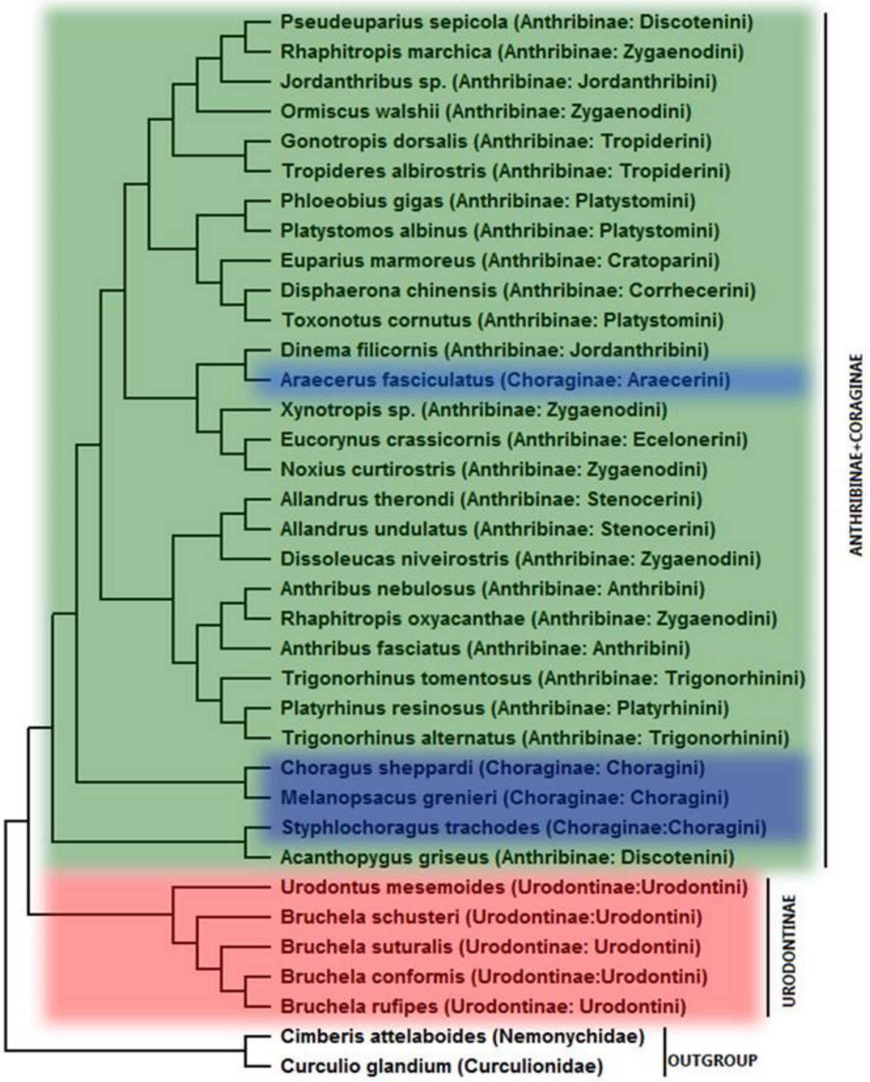

Figure 1. Evolutionary relationships within Anthribidae. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved 36 nucleotide sequences.

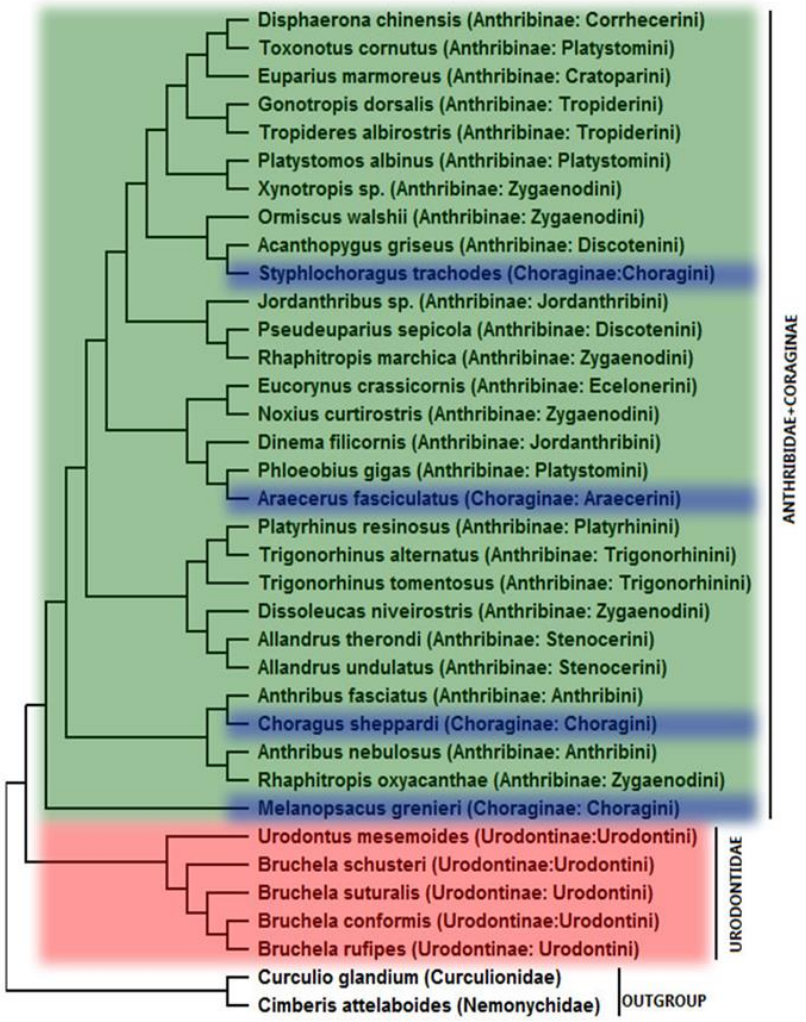

Figure 2. Evolutionary relationships within Anthribidae. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 36 nucleotide sequences.

4. Conclusion

Family Anthribidae seems to be one of the basal lineages within Curculioniodea [20]. Family level classification (subfamilies and tribes) of Anthribidae mostly depends on morphological characters and is not well resolved yet. The results of this paper show that subfamilial classification of Anthribidae needs a comprehensive revision. According to evolutionary histories presented here which inferred from COI sequences of 34 species using NJ and ML methods, members of Anthribinae + Choraginae and members of Urodontinae constitute monophyletic clades. However, the current covering of Anthribinae and Choraginae were not monophyletic, where Anthribinae was paraphyletic and Choraginae was polyphyletic. Tribal classification of Anthribidae seems to be intended for practical taxonomic purposes (i.e. morphological identification) but most of the tribes do not constitute monophyletic clades and thus not correct phylogenetically. Consequently, more phylogenetic molecular studies need to be done to resolve the current family level classification of Anthribidae.

Author Contributions

All the authors equally contributed to this work. They all read and approved the final version of the paper.

Conflicts of Interest

All the authors declare no conflict of interest.

References

[1] M. A. Alonso-Zarazaga, C. H. C. Lyal, A world catalogue of families and genera of curculionoidea (Insecta: Coleoptera) (Excepting scolytidae and platypodidae), Entomopraxis, Barcelona, 1999.

[2] R. C. Marinoni, N. G. Ganho, M. L. Monné, J. R. M. Mermudes, Hábitos alimentares em Coleoptera (Insecta), Ribeirão Preto, Holos, 2001.

[3] J. Rheinheimer, *Illustrater katalog und bibliographie der Anthribidae der welt (Insecta: Coleoptera)*, Mitteilungen des Entomologischen Vereins Stuttgart 39 (2004) 3–242.

[4] J. Mermudes, R. Leschen, *Anthribidae Billberg, 1820*, in: R. A. B. Leschen, R. G. Beutel (Eds.), Morphology and Systematics, Vol. 3 of Handbook of Zoology, De Gruyter, Berlin, 2014, Ch. 3.2, pp. 309–315.

[5] P. D. N. Hebert, A. Cywinska, S. L. Ball, J. R. deWaard, *Biological identifications through DNA barcodes*, Proceedings of the Royal Society B: Biological Sciences 270 (1512) (2003) 313–321.

[6] G. Yatkın, N. Güz, *The Use of DNA Barcoding in Entomology*, Yuzuncu Yıl University Journal of Agricultural Sciences 28 (1) (2018) 126–134.

[7] G. Kuschel, *A phylogenetic classification of Curculionoidea to families and subfamilies*, Memoirs of the Entomological Society of Washington 14 (1995) 5–33.

[8] R. A. Crowson, *On the systematic position of Bruchela Dejean (Urodon Auctt.) (Coleoptera)*, The Coleopterists Bulletin 38 (1) (1984) 91–93.

[9] B. A. Holloway, *Anthribidae (Insecta: Coleoptera)*, Fauna of New Zealand 3 (1982) 1–269.

[10] C. J. Schoenherr, Curculionidum dispositio methodica, cum generum characteribus, descriptionibus atque observationibus variis, seu Prodromus ad Synonymiae, insectorum partem IV, Fleischer, Lipsiae, 1826.

[11] P. A. Latreille, Suite et fin des Insectes, Vol. 5 of Le Règne Animal distribué d'après son organisation, pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie compare, Nouvelle édition, revue et augmentée, Déterville, Paris, 1829.

[12] T. Lacordaire, Histoire naturelle des insectes. Genera des coléoptères, ou exposé méthodique et critique de tous les genres proposés jusqu'ici dans cet ordre d'insectes, Vol. 6, Roret, Paris, 1863.

[13] T. Lacordiaire, Histoire naturelle des insectes. Genera des coléoptères, ou exposé méthodique et critique de tous les genres proposés jusqu'ici dans cet ordre d'insectes, Vol. 7., Roret, Paris, 1865.

[14] R. G. Oberprieler, 3. Curculionoidea Latreille, 1802: Introduction, Phylogeny, Vol. 3 of Handbook of Zoology, De Gruyter, Berlin, München, Boston, 2014, Ch. 3, pp. 285—300.

[15] A. E. Marvaldi, J. J. Morrone, *Phylogenetic systematics of weevils (Coleoptera: Curculionoidea): A reappraisal based on larval and adult morphology*, Insect Systematics & Evolution 31 (1) (2000) 43–58.

[16] E. Marvaldi, A. S. Sequeira, C. W. O'Brien, B. D. Farrell, *Molecular and morphological phylogenetics of weevils (Coleoptera, Curculionoidea): Do niche shifts accompany diversification?*, Systematic Biology 51 (5) (2002) 761–785.

[17] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, *MEGA X: Molecular evolutionary genetics analysis across computing platforms*, Molecular Biology and Evolution 35 (6) (2018) 1547–1549.

[18] R. C. Edgar, *MUSCLE: Multiple sequence alignment with high accuracy and high throughput*, Nucleic Acids Research 32 (5) (2004) 1792–1797.

[19] M. Kimura, *A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences*, Journal of Molecular Evolution 16 (2) (1980) 111–120.

[20] A. A. Legalov, *The first record of anthribid beetle[s] from the Jurassic of Kazakhstan (Coleoptera: Anthribidae)*, Paleontological Journal 45 (6) (2011) 629–633.