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Original article (Orijinal araştırma)

Numerical taxonomy of *Ormyrus* Westwood, 1832 (Ormyridae: Hymenoptera) species based on general morphology in Sivas[1]

Sivas İli *Ormyrus* Westwood, 1832 (Ormyridae: Hymenoptera) türleri üzerinde nümerik taksonomik çalışmalar

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Summary

The main objectives of the study were to determine the adequacy and reliability of characters used for identification of *Ormyrus* (Ormyridae: Hymenoptera) species, and the relationships between species within the genus by numerical taxonomic analysis. The specimens studied were collected in Sivas Province in 2010. Individual females of five species in to *Ormyrus* were examined. One hundred morphological characters were measured for 249 specimens. Three different numeric taxonomic analyses were performed, principal component, principal coordinate, and cluster analyses, using computer package software PAST. Principal components and coordinate analyses revealed the with characters of sufficient value to reliability distinguish five species of *Ormyrus*. Examination of the relationship between the five species by clustering analysis showed that the species belonged to two different main groups. The robustness of the currently available identification keys and new characters that could be used for of species identification was also determined.

Keywords: Chalcidoidea, numerical taxonomy, Ormyridae, *Ormyrus*

Özet

Çalışmanın asıl amacı nümerik taksonomik analiz ile Ormyrus cinsine ait türlerin teşhisinde kullanılan karakterlerin kalite ve güvenilirliğini ve türler arasındaki akrabalık ilişkilerini belirlemektir. Çalışılan örnekler 2010 yılında, Sivas ilinden toplanmıştır. *Ormyrus* Westwood (Ormyridae: Hymenoptera) cinsine ait 5 türün dişi bireyleri incelenmiştir. 249 örnek için 100 morfolojik karakter ölçülmüştür. PAST bilgisayar paket programı kullanılarak Temel Bileşenler Analizi, Temel Koordinat Analizi ve Kümeleme Analizi olmak üzere üç farklı nümerik taksonomik analiz yapılmıştır. Yapılan Temel Bileşen ve Koordinat analizleri sonucunda, belirlenen karakterlerin, *Orymrus* cinsine ait 5 türü tamamen birbirinden ayıracak yeterlilikte ve güvenilirlikte olduğu gösterilmiştir. Yapılan kümeleme analizi ile *Orymrus* cinsine ait 5 türün birbirleriyle olan akrabalık dereceleri belirlenerek, bu türlerin iki ayrı ana grup oluşturduğu gösterilmiştir. Mevcut teşhis anahtarlarının güvenilirliği ve teşhislerde kullanılabilecek yeni karakterler belirlenmiştir.

Anahtar sözcükler: Chalcidoidea, numerik taksonomi, Ormyridae, *Ormyrus*

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Introduction

Numeric taxonomy is a means of classification based on numerical principals. Briefly, it is the use numerical data within the field of systematics. Basing classification upon many characters instead of single character (not monothetic but polythetic) requires a simultaneously evaluating a range of characters. According to the Gilmour (1940) principle, the more characters are used in classification the greater the likelihood of achieving reliable results, both for the status of characters in different groups and for accurate determination of taxonomic relationships.

Taxon that are classified by numeric taxonomy are known as operational taxonomic units (OTU). All of the measurements taken on OTUs, which are to be classified in numeric taxonomy, constitute a data matrix. The most suitable geometric pattern for the data matrix is based on a spatial representation of the OTUs. Numeric taxonomy tries to reveal the hierarchical structure between the species by using clustering and permutation procedures from numerical methods (Sneath & Sokal, 1973; Podani, 1994).

The Ormyridae family, which belongs to the Chalcidoidea superfamily with the order Hymenoptera, is distinguished from related families by non-elongated cercus, metasomal tergum and the structure of ovipositor sheath on females, and consists of 153 species within three genera (Narendran, 1999; Noyes, 2017). Most species of Ormyridae are inhabitants of plants gall. Some species may be partially phytophagous, while others are either entomophagous or phytophagous (Zerova & Seryogina, 2006). *Ormyrus* is the largest and the most widespread genus, and consists of 151 species.

In the past, four different genera, *Ormyrus* Westwood, 1832, *Cyrtosoma* Perris, 1840, *Avrasyamyrus* Doğanlar, 1991 and *Ormyrulus* Boucek, 1986, were established using characters found on gaster (Doğanlar, 1991a). Doğanlar (1991b) reestablished the genus *Ormyrus* and divided the genus into three subgenera (*Monobaeus* Forster, *Tribaeus* Forster and *Crytosoma* Perris). Some authors consider that the family contains of only a single genus, *Ormyrus*. However, family has recently been seen as having three genera (Narendran, 1999; Noyes, 2017). Only the genus *Ormyrus* is recorded in Palearctic region. Several generic names have been proposed in this group, all based on the number of anelli and on the shape of gaster. Despite this proposal, and because they do not form any evident natural groups, only the genus *Ormyrus* is generally recognized (Zerova & Seryogina, 2006). Therefore, it is important to be able to identify species within the genus *Ormyrus*.

In all studies, some characters have been given more weight. Also, keys used for identification of species and characters used in these keys are limited. For this reason, it is desirable to use the numerical taxonomy as it gives equal value to all characters. The main objective of this study was to determine the adequacy and reliability of selected characters for identification of species and determination of relationships between species, and also to contributed to the development of an identification method based on numerical taxonomic analysis.

Material and Methods

Supply of material

The material of this study constituted of preserved specimens of *Ormyrus* from the collection of Entomology Museum of the Biology Department, Science Faculty, Cumhuriyet University. These specimens had been collected from Sivas Province in 2010. Five *Ormyrus* species were used and were identified using identification keys applicable to the Palearctic region (Doğanlar, 1991a, b; Hanson, 1992; Narendran, 1999; Zerova & Seryogina, 2006). The species, localities and collection dates of the specimens are given in Table 1.

Table 1. Localities and collection dates of female specimens of five *Ormyrus* species used from numerical taxonomic analysis

Taxon name	Number of specimens	Locality	Collection date
Ormyrus bingoeliensis	13	Kayapınar/Ulaş-Sivas	18/08/2010
Ormyrus cupreus	1	Sorguncuk/Merkez-Sivas	25/06/2010
O. cupreus	2	Tatlıca/Gemerek-Sivas	25/06/2010
O. cupreus	1	Karacaören/Yıldızeli-Sivas	11/07/2010
O. cupreus	1	Karaman/Divriği-Sivas	04/08/2010
O. cupreus	1	İğdeli/Kangal-Sivas	04/08/2010
O. cupreus	1	Gürpınar/Ulaş-Sivas	05/08/2010
O. cupreus	1	Elmalı/Şarkışla-Sivas	06/08/2010
O. cupreus	3	Kuluncak/Divriği-Sivas	18/08/2010
O. cupreus	4	Taşgeçit/Kangal-Sivas	18/08/2010
O. cupreus	4	Karasarbeli/Divriği-Sivas	18/08/2010
O. cupreus	1	Şenyurt/Ulaş-Sivas	19/08/2010
O. cupreus	7	Çetinkaya/Kangal-Sivas	20/08/2010
O. cupreus	6	Koşutdere/Hafik-Sivas	20/08/2010
O. cupreus	1	Eskibağ/Akıncılar-Sivas	28/08/2010
O. cupreus	35	Yellice/ Kangal-Sivas	17/09/2010
O. cupreus	8	Çetinkaya/Kangal-Sivas	17/09/2010
O. cupreus	10	Kuluncak/Divriği-Sivas	17/09/2010
O. cupreus	6	Ödek/Divriği-Sivas	17/09/2010
O. cupreus	15	Tödürge/Zara-Sivas	19/09/2010
Ormyrus orientalis	1	Kömürkaya/Şarkışla-Sivas	25/06/2010
O. orientalis	2	Taşgeçit/Kangal-Sivas	18/08/2010
O. orientalis	5	Çetinkaya/Kangal-Sivas	20/08/2010
O. orientalis	1	Yellice/Kangal-Sivas	17/09/2010
O. orientalis	1	Çetinkaya/Kangal-Sivas	17/09/2010
O. orientalis	4	Kuluncak/Divriği-Sivas	17/09/2010
Ormyrus salmanticus	1	Güllüce/Divriği-Sivas	18/07/2010
O. salmanticus	1	Koşutdere/Hafik-Sivas	20/08/2010
Ormyrus tschami	1	Karaman/Divriği-Sivas	18/07/2010
O. tschami	1	Yusufşeyh/Divriği-Sivas	04/08/2010
O. tschami	11	Ödek/Divriği-Sivas	04/08/2010
O. tschami	30	Kuluncak/Divriği-Sivas	18/08/2010

Table 1. (Continued)

Taxon name	Number of specimens	Locality	Collection date
O. tschami	19	Kasarbeli/Divriği-Sivas	18/08/2010
O. tschami	40	Taşgeçit/Kangal-Sivas	18/08/2010
O. tschami	40	Koşutdere/Hafik-Sivas	20/08/2010
O. tschami	3	Çetinkaya/Divriği-Sivas	20/08/2010
O. tschami	10	Yellice/Kangal-Sivas	17/09/2010
O. tschami	3	Kuluncak/Divriği-Sivas	17/09/2010

Specimen selection for numerical taxonomy

When selecting the specimen, the aim was to select specimens that represented the whole of the sampled locality and that exhibited intraspecific variation. By examining the female individuals of the five species, 100 morphological characters (Table 2) were measured for each of 249 specimens.

Table 2. Morphological characters used in numerical taxonomic analysis

ANTENNA	
Scape width/length	Club segment number
Pedisel width/length	Club segment width/length
Anelli 2 width/length*	Longitudinal sensilla number*
Anellus number	Longitudinal sensilla row *
Funicular segment number	Flagellum shape
Funicular segment width/length (mm)	Scape*, Pedicel*, Anelli*, Funicular segment color
FOREWING	
Submarginal/ Marginal vein	Wing width/length
Submarginal/ Postmarginal vein	Costal cell width/length
Submarginal/ Stigmal vein *	Admarginal setae number
Submarginal setae number	Basal setal line
Speculum open/ closed	Cubital setal line*
HEAD	
Head ventral width/length	Distance between toruli/distance between torulus -clypeus
Eye width/length	Distance between toruli/ distance between toruli-median ocellus
Clypeus entire	POL / OOL
Malar space length/mouth width	Head dorsal width/length *
Distance between toruli -clypeus and toruli-median ocellus	Head width / plagellum+Pedicel length

Table 2. (Continued)

THORAX	
Thorax width/length	Dorsellum width/length
Pronotum width/length	Propodeum width/length
Mesoscutum width/length	Spiracle diameter / distance to metanotum
Scutellum width/length	Thorax (Lateral) width/length
Metanotum width/length	Notauli / Axilla length
GASTER	
Carina	T6 width/length
T1 width/length	T6 color*
T1 color*	T6 sculpture
T3 width/length*	Epipygium width/length
T3 color*	Epipygium color*
T3 sculpture	Ovipositor width/length
T4 width/length	Ovipositor color*
T4 color*	Gaster (dorsal) width/length
T4 sculpture	Gaster (lateral) width/length
T5 width/length	Sercal setae 1/3
T5 color*	Sercal setae 2/4
T5 sculpture	
LEG	
Fore Coxa width/length	Tibia 1 width/length
Hind Coxa width/length	Tibia 2 width/length
Femur color*	Tibia 3 width/length
Femur 1 width/length	Spur 1 (mm) / Tibia 1 length
Femur 2 width/length	Spur 2 (mm) / Tibia 2 length
Femur 3 width/length	Spur 3 (mm) / Tibia 3 length
Tibia color*	

Results and Discussion

Five species of *Ormyrus* were determined, with *Ormyrus salmanticus* Nieves-Aldrey, 1984 being placed in two groups according to the number of anelli (one or two). Therefore 6 OTUs were recognized (Table 3).

Table 3. List of OTUs for numerical taxonomic analysis

OTU1	*Ormyrus bingoeliensis*
OTU2	*Ormyrus cupreus*
OTU3	*Ormyrus orientelis*
OTU4	*Ormyrus tschami*
OTU5	*Ormyrus salmanticus*, 1 anellus
OTU6	*Ormyrus salmanticus*, 2 anelli

Morphological characters not included in the current identification keys where chosen to improve the reliability the numeric taxonomic analysis. The identifications made from numeric taxonomical analysis were consistent with identification by existing keys. To maximize the reliability of the data care was taken to always use the same angle when taking morphological measurements. In this manner, the variations that could be result from different angles were minimized.

The 100 morphological characters used for separating OTUs from each other by principal component analysis were described from the first two components that were verifiably the highest. As shown in Table 4, variation described by components seems to reduce gradually from the first component. The first two components accounted for 95.5% of the variation. This revealed more than 90% differences between OTU based on principal component analysis (Figure 1). These results confirm the reliability of the characters chosen for numerical taxonomic analysis. Also, the effectiveness of selecting a large number of characters was confirmed. The grouping of OTUs can be seen in the graphical comparison of the first three components. In particular, it is evident that OTUs 4, 5 and 6 represent a separate group and are close to each other. The reason for this observed closeness is considered to be related to the similarity of the habitat of these species and the similarity of their hosts.

Table 4. Eigen values of the first two components measured for six operational taxonomic units and Eigen value percentages

Measured character	Eigen value	% Variance
1	1389.190	91.733
2	57.772	3.815
3	7.748	3.153

The characters listed in Table 5 are the important characters that best delineate the six OTUs (Figures 2 & 3). The characters indicated by an asterisk in Table 2 are the characters with the highest ratio for separating OTUs. These characters, especially 30, 31, 32, 54, 68, 70, 73, 76, 79, 82 and 91, when used to evaluate *O. salmanticus* with 1 or 2 anelli, indicated the possibility that these two OTUs represent two different species. It is recommended that these two OTUs be evaluated in molecular studies to assess the possibility are two different species. It is also considered that these characters are important for identification of species, that they are adequate and reliable enough to be used in identification keys. The characters listed in Table 2 are not used in existing identification keys.

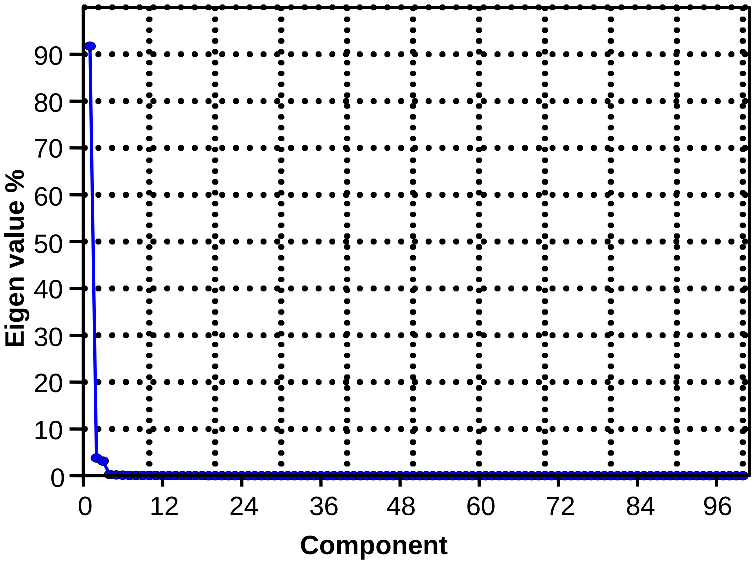

Figure 1. Percentage of Eigen values of the first two components measured for six operational taxonomic units.

Table 5. Characters values for principal components 1 and 2

Character Symbol/Number	Character name	Character values Component 1	Character values Component 2
F	Anelli (2) width/length	-0.02726	0.05653
Z	Longitudinal sensilla number	0.01476	-0.05669
27	Longitudinal sensilla row	0.05391	-0.06080
30	Scape color	0.31550	0.17640
31	Pedicel color	0.29420	-0.32350
32	Anelli color	0.29420	-0.32350
43	Head dorsal width/length	-0.02310	0.07293
47	Submarginal /stigmal vein	0.02720	0.09104
54	Cubital setal line	-0.12900	0.54860
68	T1 color	0.31550	0.17640
69	T3 width/length	-0.02922	0.07240
70	T3 color	0.31550	0.17640
73	T4 color	0.31550	0.17640
76	T5 color	0.31550	0.17640
79	T6 color	0.31550	0.17640
82	Epipygium color	-0.17330	0.45650
84	Ovipositor color	0.25190	-0.13290
91	Femur color	0.31550	0.17640
92	Tibia color	-0.09597	0.05062

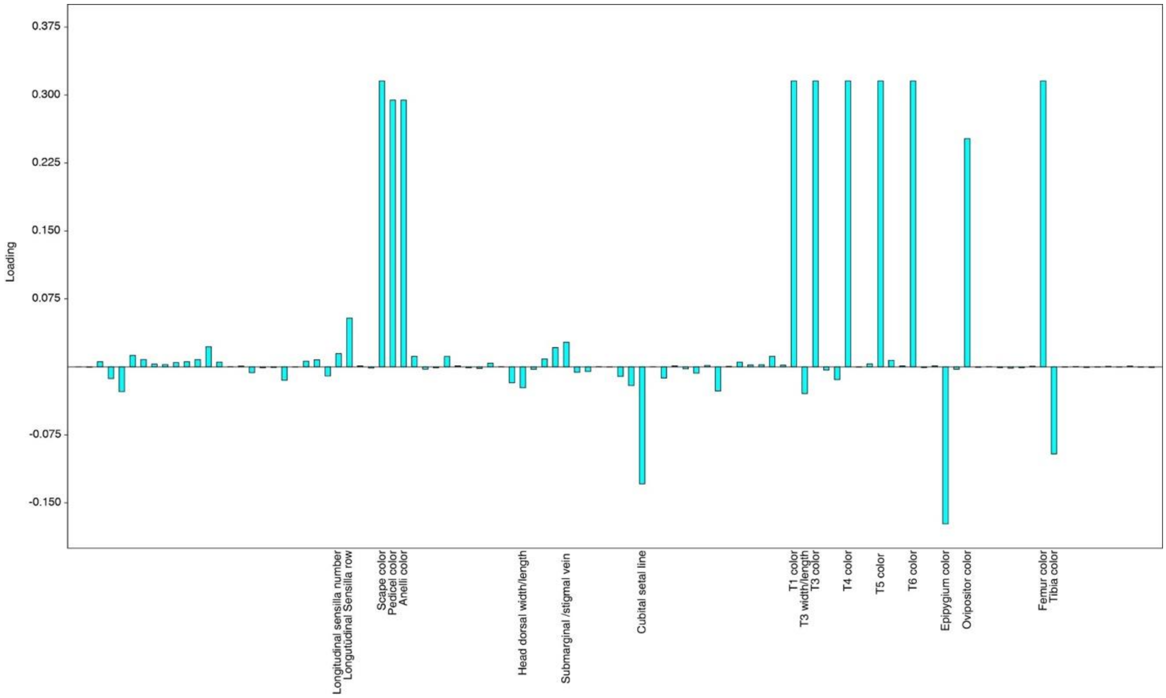

Figure 2. The first principal component graphic of measured characters.

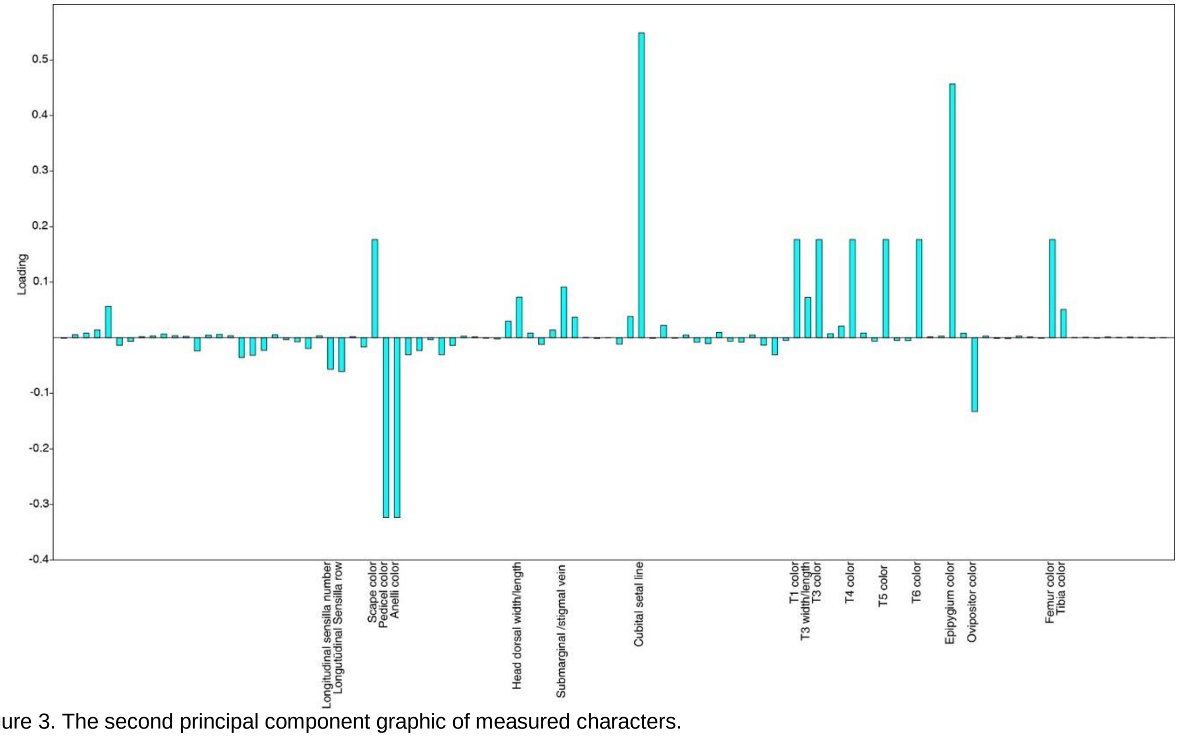

Figure 3. The second principal component graphic of measured characters.

The Eigen values of the first two components from the principal coordinate analysis explain more than 90% difference between the OTUs. In Table 6 it is evident that the amount of variation explained by the coordinates from the principal component analysis, reduce gradually from the first coordinate and describe 95.54% of the variation of the first two coordinates. The characters which revealed 95.54% of the variation at OTUs seem to be highly effective in separating of OTUs (Figure 4).

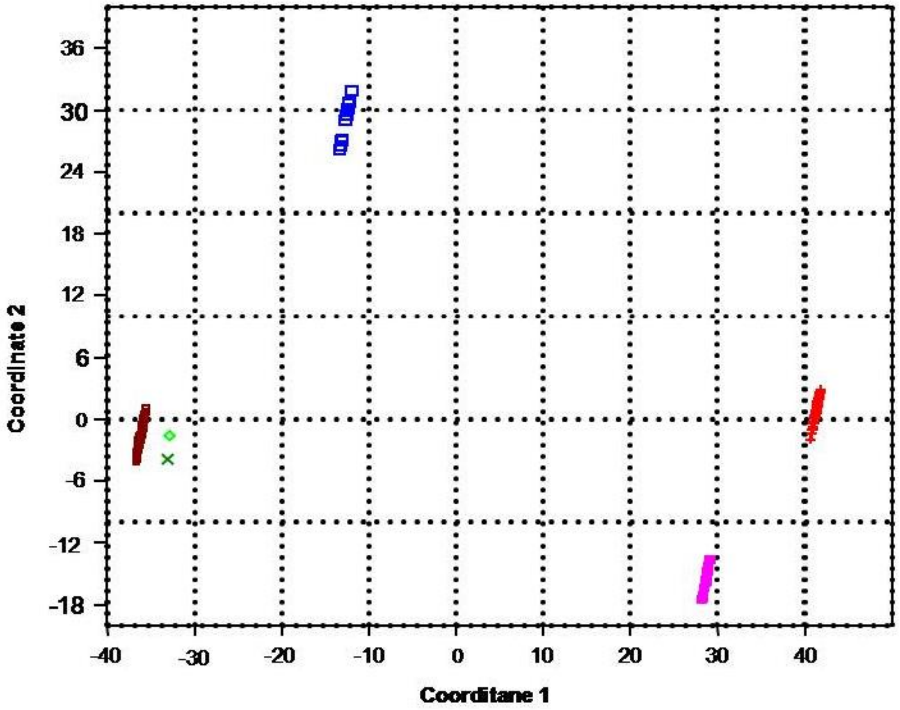

Figure 4. Distance and locations of six operational taxonomic units the first two coordinates.

In the unweighted pair group method dendrogram (Figure 5) from the cluster analysis the OTUs were put in two major groups. *Ormyrus bingoeliensis* Doğanlar, 1991 and *Ormyrus cupreus* Askew, 1998 are close to each other and in a separate group. Also, *Ormyrus orientalis* Walker, 1871 and *Ormyrus tschami* Doğanlar, 1991 are close to each other and in a separate group. *Ormyrus salmanticus* (1 anellus), *O. salmanticus* (2 anelli) and *O. orientalis* are relatively close to each other and in a separate group.

Ormyrus salmanticus (1 anellus) and *O. salmanticus* (2 anelli) are the closest taxons to each other and in a separate group (Figure 5). According to principal components, principal coordinate and cluster analyses, *O. salmanticus* (1 anellus) and *O. salmanticus* (2 anelli) could potentially be regarded as two distinct species. However, given that only two specimens of *O. salmanticus* were available for the present study, it is recommended that molecular analysis be used to determine the status of these two OTUs.

The results of all these analyses support the adequacy and reliability of the morphological characters selected for distinguishing the five species. The classification obtained by numerical taxonomy seem to be compatible with identification made using existing identification keys (Doğanlar, 1991a,b; Hanson, 1992; Zerova & Seryogina, 2006; Narendran, 1999; Gençer, 2014). The analyses performed were quite efficient for distinguishing the species. The closeness of species to each other was explained and it is suggested that the selected characters should be considered for inclusion in any future identification keys for this genus.

Table 6. Eigen values of the first two coordinates measured for six operational taxonomic units and Eigen value percentages

Measured character	Eigen value	% Variance
1	34452	91.733
2	14327	3.815
3	11842	3.153

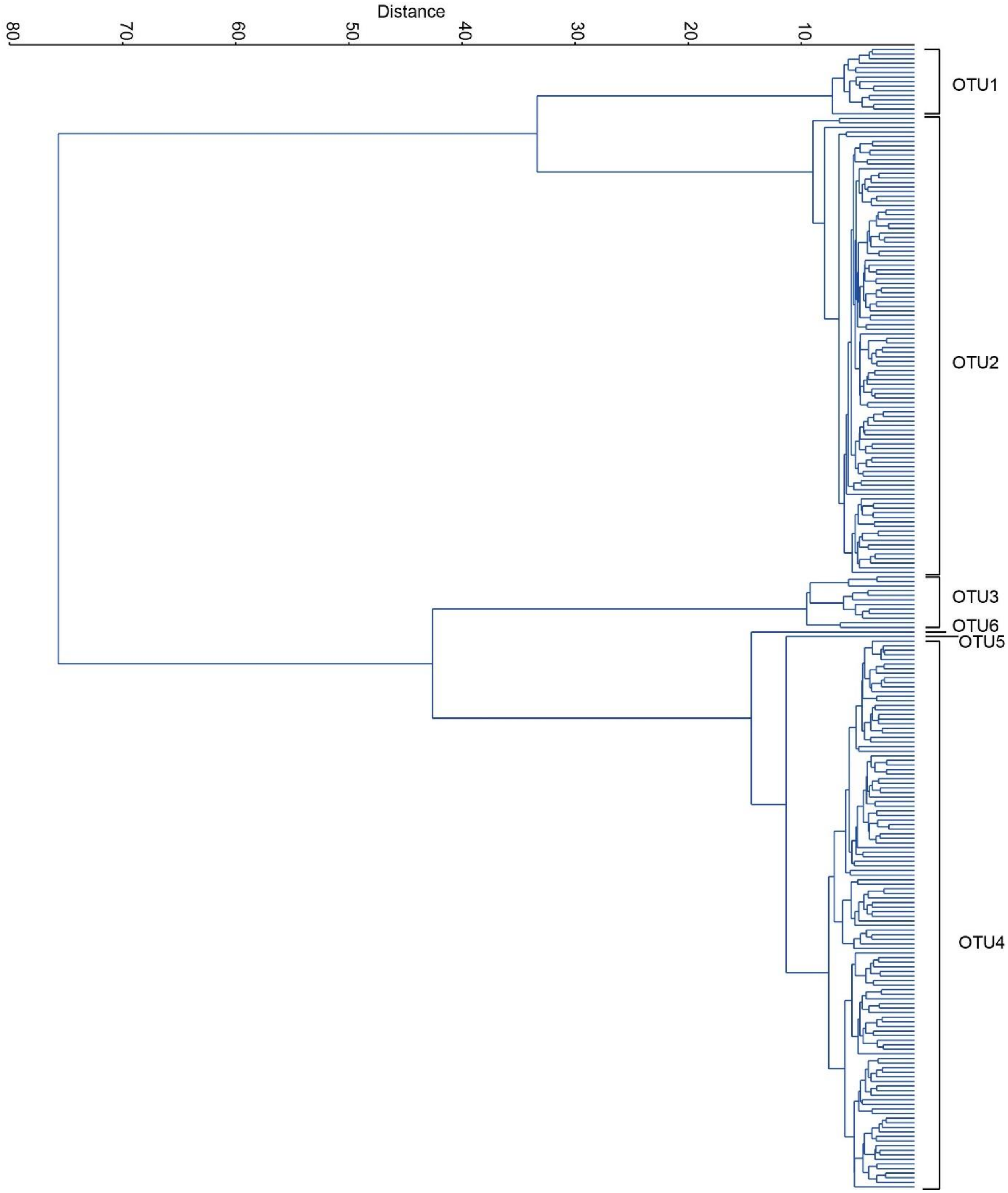

Figure 5. Distance tree created by clustering method for six operational taxonomic units.

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