Some *Assyriella* Species in South East Anatolia in Turkey Differentiated by Multivariate Statistical Analysis (Gastropoda: Pulmonata, Helicidae)

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Abstract: This study was performed on 3 species of the genus *Assyriella* (*A. escheriana*, *A. guttata* and *A. thospitis*) collected from Diyarbakır province in Southeast Anatolia. Two derived and 6 shell variables of 50 specimens were examined for each species. Hierarchical clustering and discriminant analysis were used to evaluate the data in order to clarify and contribute to some systematic problems of these species.

It was shown that Ward's method with Euclidean distance is the most appropriate hierarchical clustering method to cluster these species. *A. escheriana* is clearly differentiated from *A. guttata* and *A. thospitis* by both Ward's method and discriminant analysis. The results of discriminant analysis and Ward's method reveal that *A. thospitis* and *A. guttata* may have a close evolutionary relationship. Also, the most important shell characters (variables) that differentiate these species from each other were determined by discriminant analysis and the success efficiency of these techniques in systematic problems was observed.

Consequently, the results of this study showed that more precise identification can be made in classification of *A. escheriana, A. guttata* and *A. thospitis* if classical criteria are used together with the group classification functions obtained from discriminant analysis.

Key Words: Assyriella, Biometry, Numeric Taxonomy

Türkiye, Güneydoğu Anadolu Bölgesi'nde Bulunan Bazı *Assyriella* Türlerinin (Gastropoda, Pulmonata, Helicidae), Çok Değişkenli İstatistik Metodlarla Ayırımının Yapılması

Özet: Bu çalışma, Güneydoğu Anadolu Bölgesi, Diyarbakır ili civarından toplanmış *Assyriella* genusunun üç türü (*A. escheriana , A. guttata* ve *A. thospitis*) üzerinde gerçekleştirilmiştir. Her bir tür için 50 örnek üzerinde, iki türetilmiş ve altı kabuk değişkeni incelendi. Bu üç türün bazı sistematik problemlerinin çüzümüne katkıda bulunmak amacıyla, veriler, hiyerarşik gruplama ve diskriminant analizi teknikleri ile değerlendirildi ve bu tekniklerin sistematikte ne derecede başarılı olarak kullanılabilecekleri gözlendi.

Öklit uzaklığı kullanılarak, Ward metodunun bu türleri gruplamada daha uygun bir hiyerarşik gruplama metodu olduğu görülmüştür. Hem Ward metodu hem de diskriminant analizi *A. escheriana'yı A. guttata* ve *A. thospitis*'ten belirgin bir şekilde ayırmaktadır. Diskriminant analizi ve Ward metodunun sonuçları, *A. guttata* ve *A. thospitis*'in evrimsel olarak daha yakın akraba olabilecekleri göstermektedir. Ayrıca, bu türleri biribirinden ayıran en önemli kabuk karakteristikleri (değişkenleri) diskriminant analizi ile belirlenmiş ve bu tekniklerin sistematikteki etkinlikleri gözlenmiştir.

Sonuç olarak, bu çalışmanın bulguları *A. escheriana, A. guttata* ve *A. thospitis*'in sınıflandırılmasında, geleneksel kriterler, diskriminant analizinden elde edilen grup sınıflandırma fonksiyonları ile birlikte kullanılırsa, daha doğru teşhislerin mümkün olabileceğini göstermektedir.

Anahtar Sözcükler: Assyriella, Biyometri, Numerik Taksonomi

Introduction

Schütt (1) stated that the species of the genus *Assyriella* spread in the Middle East, Near East and some islands such as Rhodes and Cyprus originally developed in Southeastern Anatolia. Their classification is mainly based

on shell features and genital apparatus. Recently, a revision was made on the species of this genus. A total of 2 new and 15 known species have been described by Schütt and Subai (2).

Due to morphological and anatomical similarity, *Assyriella* species have many synonyms. For instance, *Assyriella thospitis* (Schütt and Subai, 1996) which was described as a new species in 1996 (2), was previously considered to be *Helix ergilensis* by Galland in 1885. *Assyriella guttata* (Olivier 1804) and *Assyriella escheriana* (Bourguignat, 1864) were considered to be *Helix guttata* by Olivier in 1804 and *Helix escheriana* by Bourguignat in 1864, respectively.

The shell features of *A.guttata* and *A.escheriana* and the comparative anatomy of their reproduction systems were studied by Agüloğlu and Balcı (3).

The present study was carried out on 3 species of the genus *Assyriella, A. escheriana, A. guttata* and *A. thospitis*, collected from Diyarbakır province in Southeastern Anatolia in order to clarify and contribute to the taxonomic status of these species by employing hierarchical clustering and discriminant analysis techniques and the efficiency of these techniques was observed. Some shell characters (variables) were used in the multivariate statistical techniques since these characters are the basis of the original species description.

Materials and Methods

Materials

This study was performed on the specimens of 3 species (*A. escheriana, A. guttata, A. thospitis*), collected from Diyarbakır province in Southeast Anatolia in Turkey between 1995 and 1999 and identified according to the traditional taxonomic characters used by Schütt and Subai (2). These specimens are kept at the Dicle University Science Faculty Museum (DUM). Fifty specimens were randomly drawn for each of the 3 species. The selected specimens of *A. escheriana A. guttata*, and *A. thospitis* were numbered [1-50], [51-100] and [101-150], respectively.

Methods

Box plots

Box plots provide a simple graphical summary of a group (groups) of cases defined by a categorical (grouping) variable. Tukey (4) originally presented them as schematic plots. Vellman and Hoalgin (5) introduced

them to non-technical users and demonstrated their power for a range of data. McGill et al. (6) implemented confidence intervals on the medians of several groups in box plots. If the intervals around 2 medians do not overlap, one can be about 95 % confident that the population medians are different and so the confidence intervals are useful for judging difference between groups. More information about constructing and interpreting box plots can be found in Hamilton (7).

Hierarchical clustering

There are many clustering methods and similarity coefficients. Many of them can be found in Sneath and Sokal (8), Hartigan (9); Abbot et al. (10) and Everit (11).

Discriminant Analysis

Discriminant functions were developed independently by Fisher (12), Mahaonobis (13) and Hotelling (14), whose primary interests were in different areas. The theoretical base of this analysis can be found in detail in Chatfield and Colins (15), Johnson and Wichern (16) and in the publications dealing with multivariate statistics. In summary, discriminant analysis is a technique for finding functions so as to discriminate several groups previously defined. It is therefore of considerable interest to those wishing to classify specimens, on each of which a number of measurements have been made and which are to be collected in previously defined groups. This analysis was employed for the same purpose in this study.

A large number of discriminant analysis studies and hierarchical clustering applications have been described such as in Akbayın et al. (17), Togan et al. (18), Özbay and Akbayın (19), Kristensen and Christensen (20), and Mukaratirva et al. (21).

Measurements

Two derived and 6 shell variables shown in Figure 1 and Table 1 were measured on the selected 150 specimens in millimeters by a calliper compass. In this way, we obtained a 150x8 data 3 with three categories. The data matrix with descriptive statistics by categories is given in Table 2. STATISTICA (22) vwas used for the statistical analysis.

Descriptive statistics, hierarchical clustering methods and discriminant analysis were used to evaluate the data matrix in order to clarify some systematic problems of these species.

Table 1. Measured variables of Assyriella species

| Shell variable (Character) | Abbreviation |
|--|--------------|
| Shell Diameter (mm) | ShD |
| Shell Height (mm) | ShH |
| Ratio of shell diameter to shell height | ShD/ShH |
| Aperture Height (mm) | ApH |
| Aperture Width (mm) | ApW |
| Ratio of Aperture Height to aperture width | ApH/ApW |
| Spire Height (mm) | SpH |
| Truncation Width (mm) | TrW |

Results

The multiple box plot given in Figure 2 was used to determine the classificatory values of the measured characteristics of *Assyriella* species in question. Therefore, the data matrix was categorised with respect to the species.

If Figure 2 is reviewed, the variables ShD/ShH, ApH/ApW and TrW have no classificatory value because of the approximately equal distribution of these variables. So these variables were removed when the cluster analysis was performed. On the other hand, the remaining variables have more or less classificatory value.

In the present study, the hierarchical methods with different similarity coefficients found in the STATISTICA (22) clustering module were applied to the data matrix given in Table 2 by removing the variables (ShD / ShH, ApH / ApW and TrW) that have no classificatory values. The results of these operations were observed. During the process, it was determined that Ward's (23) method

with Euclidean distance is a more appropriate method than the others due to its producing clusters at high similarity level. The dendrogram obtained by Ward's method is given in Figure 3.,

The data matrix given in Table 2 was subjected to discriminant analysis taking species as grouping variables. Eigen values and discriminant functions associated with these eigen values were obtained. These functions were tested by chi-square statistics. The results of these operations are given in Table 3. The test statistics shown that the 2 discriminant functions were statistically significant (p< 0.001) in discrimination of the 3 species.

The scatter plot of the first canonical variate (CV1) by the second canonical variate (CV2) is presented in Figure 4.

The group classification functions are used to determine to which group (category) a new specimen most likely belongs. A specimen is classified into the group for which it has the largest classification function score. The group classification functions are given in Table 4.

Group classification functions were calculated for the data matrix (see Table 2) to see how our specimens are classified by these functions. Observed memberships against those predicted by these functions are given in Table 5.

Canonical loadings are correlations between variables and discriminant functions. These loadings are used to determine how much and in which direction a variable contributes to discrimination. The canonical loading produced during the application of discriminant analysis are given in Table 6 in decreasing order of importance.

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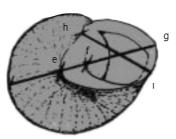


Figure 1. The location where measurements of shell variables were taken on each specimen of *Assyriella*. ab: Shell Height (ShH) ac: Spire Height (SpH) dg: Shell Diameter ShD) ef: Truncation Width (TrW) eg: Aperture Width (ApW) hı: Aperture Height (ApH)

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | l | |
|-----------------------------|----------|------------|-------|----------------|--------------|------------|-------|-------|-------|------------|-------|-------|--------------|----------------------|--------|-------|-------|-------|------------|-------|-------|-------|-------|--------------|--|------------|-------|------------|-------|-------|--------|------------|----------------------------|----------|-------|-------|-------|--------------|----------------------|---|-------|-------|------------|------------|------------|-------|-------------|--|
| Trw | 5.3 2 | 2.3 | 2.6 | 4 0 7 0 | 0.7 | 1 2 | 24 | 2.4 | 2.4 | 2.1 | 2.2 | 2.6 | 2.6 | 0.2 | 7 T C | 2.2 | 2.3 | 2.2 | 2.3 | 1.8 | 2.3 | 2.3 | 2.4 | 2.2 | | 5 0 | 2.1 | 2.1 | 2.3 | 2.6 | 2.5 | 2.2 | 1.2 | t 23 | 2.1 | 2.1 | 2.4 | 1.1 | 0 4 | 1 C | 2.4 | 2.0 | 2.3 | 2.4 | 2.3 | 2.27 | 0.178 | |
| / SpH | 16.0 | 17.2 | 15.7 | 15.3 | 17.0 | 17.3 | 16.7 | 15.4 | 16.3 | 14.9 | 16.6 | 16.2 | 15.4 | 1./1 | 0.01 | 16.6 | 17.3 | 14.9 | 16.0 | 16.1 | 17.5 | 14.7 | 16.5 | 15.8 | 1.01 | 17.2 | 15.6 | 16.4 | 16.2 | 16.9 | 16.0 | 16.0 | 15.1 15.8 | 16.5 | 16.4 | 16.5 | 17.0 | 15.7 | 0.01 | 16.9 | 16.2 | 17.4 | 16.1 | 16.4 | 16.2 | 16.29 | 0.770 | |
| ApH/ApW | 1.274 | 1.020 | 0.967 | 1.138 0.07E | 1141 | 1 036 | 0.975 | 1.200 | 0.995 | 1.108 | 0.953 | 1.168 | 1.068 | 1.033 | 1 106 | 1.150 | 1.057 | 1.229 | 1.098 | 1.078 | 1.034 | 0.966 | 1.177 | 1.197 | 1.109 | c00.1 | 1.036 | 1.109 | 1.069 | 1.061 | 1.204 | 066.0 | 1.064 | 1.051 | 1.035 | 1.079 | 0.990 | 0.990 | 1111 | 1001 | 1.123 | 1.048 | 1.083 | 1.121 | 1.041 | 1.07 | 0.076 | |
| ApW | 16.8 | 20.5 | 21.2 | 18.1 | 19.1 | 1.61 | 20.0 | 18.0 | 20.5 | 18.5 | 21.1 | 17.3 | 19.2 | 1.12 a tc | 0.12 | 18.0 | 19.3 | 17.9 | 19.4 | 19.2 | 20.3 | 20.7 | 18.1 | 17.3 | C./1 | 19.3 | 19.3 | 19.2 | 18.9 | 19.7 | 18.1 | 20.2 | 18.7 10 E | 19.5 | 19.9 | 19.0 | 19.4 | 20.6 | 10.9 | 10.9 | 17.9 | 18.7 | 19.2 | 20.7 | 19.3 | 19.29 | 1.118 | |
| ApH | 21.4 | 20.9 | 20.5 | 20.6 | 21.8 | 20.4 | 19.5 | 21.6 | 20.4 | 20.5 | 20.1 | 20.2 | 20.5 | 511.8 1 1 C | 20.9 | 20.7 | 20.4 | 22.0 | 21.3 | 20.7 | 21.0 | 20.0 | 21.3 | 20.7 | 19.4 | 21.2 | 20.0 | 21.3 | 20.2 | 20.9 | 21.8 | 20.0 | 19.9 10.8 | 20.5 | 20.6 | 20.5 | 19.2 | 20.4 | 012 012 | 19.7 | 20.1 | 19.6 | 20.8 | 23.2 | 20.1 | 20.65 | 0.775 | |
| A. thospitis ShH ShD/ShD | | 17.7 2.051 | | 18.2 2.000 | | | | | | 17.8 2.022 | | | 17.5 2.063 | | | | | | 17.1 2.076 | | | | | | 10.0 2.090 17.6 2.045 | 18.6 1.882 | | 17.8 1.944 | | | | 17.7 2.073 | 18.9 1.915 177 1.066 | | | | | | 17.8 2.064 | | | | 17.3 2.046 | 17.7 1.960 | 17.8 2.028 | | 0.602 0.078 | |
| A. ShD | 35.4 | 36.3 | 36.6 | 36.4 | 365 | 36.4 | 37.7 | 38.2 | 35.6 | 36.0 | 36.2 | 36.6 | 36.1 | 20.J | 0.10 | 37.3 | 35.6 | 36.5 | 35.5 | 36.7 | 36.1 | 37.5 | 36.9 | 34.9 | 04.0 | 35.0 | 36.8 | 34.6 | 37.8 | 34.9 | 35.8 | 36.7 | 30.2 3.1 B | 36.5 | 37.9 | 35.1 | 37.0 | 39.2 | 1./5 | 35.7 | 35.2 | 36.5 | 35.4 | 34.7 | 36.1 | | 1.095 | |
| S | 101 | 102 | 103 | 104 10F | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | ۲۱ م ۱۱ م | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 121 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 130 | 138 | 139 | 140 | 141 | 142 | 041 | 145 | 146 | 147 | 148 | 149 | 150 | | | |
| TrW | 2.4 | 2.6 | 2.0 | 0 7 | - 6 | 44 | 5.3 | 2.6 | 2.2 | 2.6 | 2.2 | 2.4 | 2.5 | 4 5 | 1 C | 12 | 2.5 | 2.4 | 2.1 | 2.1 | 2.3 | 2.3 | 2.2 | 5.2 | 5. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. | 53 | 2.3 | 2.6 | 2.3 | 2.1 | 2.2 | 2:0 | 9 Y C | 2.2 | 2.1 | 2.1 | 2.2 | 2.2 | 0.0 | 212 | 54 | 2.6 | 2.6 | 2.1 | 2.3 | 2.30 | 0.204 | |
| SpH | 15.6 | 15.7 | 16.0 | 16.4 | 16.7 | 16.6 | 15.4 | 15.9 | 16.2 | 16.3 | 16.0 | 17.2 | 15.6 | 15.0 | 14.6 | 16.1 | 16.5 | 16.4 | 16.6 | 15.5 | 16.0 | 16.4 | 15.9 | 16.5 | 16.2 | 16.5 | 16.0 | 16.6 | 16.6 | 16.1 | 15.9 | 16.1 | 10.5 | 16.6 | 16.6 | 15.8 | 15.1 | 16.8 | 5.01 | 15.9 | 16.7 | 16.6 | 15.8 | 15.8 | 16.3 | | 0.501 | |
| ApH/ApW | 1.046 | 1.186 | 1.220 | 1.011 | 1 201 | 1 011 | 1.162 | 1.051 | 1.195 | 1.123 | 1.280 | 1.016 | 1.126 | 2/1.1 | 1 094 | 1.134 | 1.140 | 1.038 | 1.175 | 1.058 | 1.274 | 1.115 | 1.138 | 1.319 | 1 211 | 1.135 | 1.327 | 1.105 | 1.137 | 0.975 | 1.161 | 0.985 | 1.129 | 1.100 | 1.149 | 066.0 | 1.311 | 1.151 | col 1 | 1 156 | 1.127 | 1.060 | 1.038 | 1.141 | 1.342 | 1.14 | 0.091 | |
| ApW A | 19.5 | 17.2 | 16.4 | 17.5 | 17.4 | 186 | 17.3 | 19.7 | 18.5 | 17.9 | 16.1 | 19.1 | 18.3 | 15.0 | 180 | 18.7 | 17.8 | 18.2 | 17.1 | 19.1 | 16.8 | 18.2 | 18.8 | 16.6 | 0.71 | 17.8 | 16.2 | 19.0 | 16.8 | 20.3 | 17.4 | 19.7 | 17.0 | 18.0 | 18.1 | 19.9 | 16.1 | 18.5 | 10.1 | 17.9 | 18.1 | 20.0 | 18.5 | 17.7 | 16.1 | | 1.126 | |
| A. guttata ApH | 20.4 | 20.4 | 20.0 | 17.7 | 0.13 | 18.8 | 20.1 | 20.7 | 22.1 | 20.1 | 20.6 | 19.4 | 20.6 | 20.2 | 19.7 | 21.2 | 20.3 | 18.9 | 20.1 | 20.2 | 21.4 | 20.3 | 21.4 | 21.9 | 20.7 | 20.2 | 21.5 | 21.0 | 19.1 | 19.8 | 20.2 | 19.4 | 20.4 | 19.8 | 20.8 | 19.7 | 21.1 | 21.3 | 10.02 | 20.7 | 20.4 | 21.2 | 19.2 | 20.2 | 21.6 | | 0.873 | |
| A. ShD/ShH | 2.057 | 1.939 | 2.090 | 2.000 | 2.046 | 2.081 | 2.077 | 1.982 | 1.983 | 2.040 | 2.065 | 1.949 | 2.042 | 201.2 | 1 977 | 2.302 | 1.919 | 1.955 | 2.054 | 1.994 | 2.080 | 2.230 | 1.953 | 2.117 | 021.2 | 2.034 | 2.085 | 1.971 | 1.944 | 1.972 | 2.138 | 1.879 | 2.104 | 2.091 | 2.000 | 2.053 | 2.011 | 2.077 | 241.2 241.2 | 2 023 | 2.132 | 1.917 | 2.114 | 2.065 | 1.994 | | 0.087 | |
| ShH | 17.6 | 17.9 | 16.6 | 17.4 | 10.1 | 2.71 | 16.8 | 16.9 | 17.4 | 17.3 | 16.8 | 17.6 | 16.8 | 17.4 | 17.2 | 16.2 | 18.6 | 17.7 | 16.8 | 18.0 | 17.4 | 16.1 | 17.1 | 17.1 | 16.0 | 17.5 | 16.5 | 17.5 | 17.8 | 17.9 | 16.7 | 18.2 | 10.3 16.3 | 16.4 | 17.6 | 16.9 | 17.5 | 16.8 | 10.1 | 17.3 | 16.7 | 18.0 | 16.6 | 17.0 | 17.8 | 17.15 | 0.597 | |
| ShD | 36.2 | 34.7 | 34.7 | 877.8 | 0.45 7.47 | 1 8 55 | 34.9 | 33.5 | 34.5 | 35.3 | 34.7 | 34.3 | 34.3 27.0 | 2.02 2.02 1.02 | - 77 | 37.3 | 35.7 | 34.6 | 34.5 | 35.9 | 36.2 | 35.9 | 33.4 | 36.2 21 1 | 0.00 26.0 | 35.6 | 34.4 | 34.5 | 34.6 | 35.3 | 35.7 | 34.2 | 27 27 27 27 27 | i M M | 35.2 | 34.7 | 35.2 | 97.9 | 0.47 0.13 0.13 | 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1 | 35.6 | 34.5 | 35.1 | 35.1 | 35.5 | 34.97 | 0.806 | |
| S | 51 | 22 | ß | 27 H | 8 8 | 3 6 | 8 | 20 | 8 | 61 | 82 | 8 | 25 | 8 % | 3 6 | 6 88 | 60 | 70 | 7 | 72 | 5 | 74 | 花 | 19 P | 1 02 | 0 62 | 8 | 81 | 82 | 8 | 28 | 83 | 86 | 6 8 | 88 | 06 | 91 | 26 2 | 8 9 | 1 8 | 8 | 97 | 86 | 66 | 100 | | | |
| TrW | 22 | 1.7 | 1.4 | 1.7 | e. 1 | <u>, e</u> | 1.7 | 1.6 | 2.0 | 1.7 | 2.0 | 1.8 | 1.8 | 0.7 | ο σ | 5.3 | 1.6 | 1.2 | 1.9 | 1.4 | 2.2 | 1.7 | 1.5 | 1.7 | - 0 | 1.8 | 1.6 | 1.6 | 2.3 | 1.5 | 1.9 | 2.1 | 5.7 | t 6. | 1.4 | 1.8 | 1.3 | с. I с. I | | 17 | 2.2 | 1.9 | 1.5 | 2.1 | 2.3 | 1.80 | 0.296 | |
| SpH | 10.6 | 12.0 | 14.1 | 10.9 | 14.0 | 11 7 | 11.5 | 11.8 | 13.0 | 14.4 | 12.9 | 12.3 | 14.2 | 0.51 | 14.4 | 15.3 | 14.5 | 12.0 | 11.4 | 13.3 | 14.3 | 12.7 | 10.3 | 12.4 | 0.21 | 12.5 | 14.2 | 13.2 | 16.4 | 12.9 | 12.1 | 12.9 | 14.8 | 13.8 | 12.9 | 10.3 | 14.7 | 12.4 | 0.5 | 11.6 | 15.3 | 11.1 | 13.1 | 10.3 | 11.8 | 12.88 | 1.537 | |
| A. escheriana ApH/ApW | 1.425 | 1.267 | 1.200 | 1.381 | 0.815 | 1 076 | 0.877 | 1.078 | 1.528 | 1.145 | 1.076 | 1.047 | 1.289 | CU2.1 | 1 245 | 1.028 | 0.916 | 0.953 | 0.886 | 1.247 | 1.423 | 1.139 | 0.983 | 1.000 | 701.1 | 0.892 | 1.218 | 1.515 | 1.170 | 1.110 | 1.282 | 1.231 | 1.011 | 1.144 | 1.214 | 1.384 | 1.253 | 1.301 | 1.100 | 1 333 | 0.867 | 1.024 | 0.901 | 0.744 | 1.272 | 1.14 | 0.191 | |
| ApW A. | 15.3 | 14.6 | 17.0 | 16.8 | 16.2 | 18.4 | 20.4 | 16.7 | 12.5 | 15.9 | 17.0 | 17.2 | 16.6 | 16.2 | 14.7 | 17.7 | 17.9 | 17.0 | 16.6 | 15.0 | 14.2 | 16.5 | 17.7 | 20.0 | 0.01 | 203 | 17.4 | 13.0 | 15.3 | 14.5 | 14.9 | 16.9 | 0.11 | 17.4 | 17.3 | 15.1 | 15.4 | 16.6 | 0.01 | 15.0 | 16.5 | 16.9 | 17.1 | 15.6 | 15.1 | 6.35 | 1.665 | |
| ApH | | 18.5 | 20.4 | | | | | | | | | | 21.4 | | | | | | | | 20.2 | | | | | 18.1 | | | | 16.1 | | | 17.8 | | | | | 21.6 | | 0.02 | 14.3 | | | | | | 2.373 1 | |
| Hus/Dus | 3.290 | 2.424 | 2.738 | 1.861 2 11E | 2,603 | 000.1 | 1.965 | 1.981 | 1.901 | 2.241 | 2.225 | 2.713 | 2.819 | 067.2 | 1 608 | 2.278 | 2.760 | 3.184 | 2.248 | 2.147 | 2.557 | 2.144 | 2.688 | 3.606 | 2.100 2.16E | 2.143 | 2.465 | 2.630 | 2.553 | 1.950 | 2.511 | 1.800 | 2 dd.2 | 1.787 | 2.878 | 2.277 | 1.993 | 2.045 | 040.1 | 1 867 | 2.200 | 3.367 | 2.813 | 1.826 | 2.036 | | 0.455 | d Figure 1. |
| ShH | | | 12.2 | | | | | | | | | | 11.6 | | | | | | | | 12.2 | | | | 0.01 | | | 12.7 2 | | | 13.7 2 | | | | | | | | | | | | | | | | 1.922 (| Table 1 an |
| ShD | 30.6 | 32.0 | 33.4 | 32.2 | 34 1 34 1 | - og | 28.3 | 31.7 | 30.8 | | | 33.1 | | 0.2C 1 22 1 | | | 35.6 | 32.8 | 31.7 | 29.2 | 31.2 | 32.8 | 29.3 | | | 30.05 | | | | | 34.4 | 27.9 | 33.0 33.0 | 25.2 | 35.4 | 32.1 | 28.7 | 27.2 | 21.0 | 28.0 28.0 | 31.9 | 36.7 | 34.6 | 29.4 | 27.9 | | | *For abbreviation see Table 1 and Figure |
| S | _ | ~2 | т | 4 4 | n u | 0 | | თ | 10 | Ξ | 12 | 13 | 14 | 0 ¥ | 51 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 20 | 17 | 0 62 | 0e | 31 | 32 | 33 | 34 | 35 | 95 75 | 6 8 | 39 | 40 | 41 | 42 | 4 F | 4 4 | 46 | 47 | 48 | 49 | 50 | Mean | ÷ | *For abbr |

Table 2. Data matrix obtained from the randomly selected specimens.*

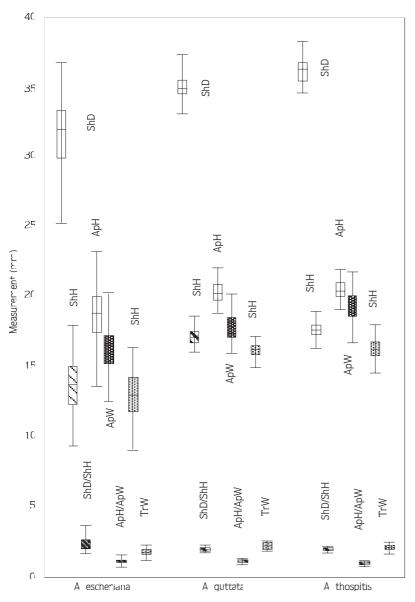


Figure 2. Multiple box plot of the measured variables by *Assyriella* species.

*For abbreviation see Table 1 and Figure 1.

Discussion

Ward's method with Euclidean distance based on these variables is the most appropriate hierarchical clustering method for these species (*A. escheriana, A. guttata, A. thospitis*) because it produces clusters at high similarity level and the obtained clusters are in accord with the classification determined by the traditional method (See Figure 2 and Table 2).

Clusters I, II and III were constituated at an 87.5 % similarity level by Ward's method. Cluster I, which includes specimens of *A. escheriana*, is clearly

differentiated from Clusters II and III, which include *A. thospitis* and *A. guttata* respectively. However, some specimens, which were originally described as *A. thospitis* or *A. guttata*, fall into the same clusters. So there may be an evolutionarily close relationship between *A. guttata* and *A. thospitis* rather than *A. escheriana* (See Figure 3).

In the case of a similarity level greater than 87.5 %, Cluster I is segregrated into 2 sub- clusters. This result is interpreted as *A. escheriana* possibly comprising 2 varieties. The impression of 2 varieties may be considered a subject for new research (Figure 3).

Table 3. Raw coefficients and constants for Canonical Variables.*

| Variable | CV1 | CV2 | | |
|----------|---------------|------------|------------|---------|
| ShD | 0.0693 | 0.3505 | | |
| ShH | -1.2464 | -0.3207 | | |
| ShD/ShH | -4.2137 | -2.4573 | | |
| ApH | -0.5160 | -1.4631 | | |
| ApW | 0.1105 | 2.1397 | | |
| ApH/ApW | 5.2743 | 24.8986 | | |
| SpH | -0.5799 | -0.4077 | | |
| TrW | -1.1122 | -1.5015 | | |
| Constant | 40.3478 | -29.2369 | | |
| | Explained | Cumulative | Chi-squre | P-level |
| | variance | percent of | Statistics | |
| | (Eigen value) | explained | | |
| | | variance | | |
| | 9.7390 | 0.9792 | 367.69125 | 0.00000 |
| | 0.2073 | 1.0000 | 27.03905 | 0.00033 |
| | | | | |

*For abbreviation see Table 1 and Figure 1.

CV1: The first canonical variable, CV2: The second canonical variable

Table 4. Group classification functions and constants.*

| Variable | A. escheriana | A. guttata | A. thospitis |
|----------|---------------|------------|--------------|
| ShD | -69.87 | -70.52 | -70.21 |
| ShH | 201.60 | 209.26 | 210.21 |
| ShD/ShH | 1080.85 | 1107.68 | 1109.38 |
| ApH | -251.28 | -247.20 | -248.27 |
| ApW | 290.38 | 288.27 | 290.50 |
| ApH/ApW | 4344.68 | 4296.30 | 4318.04 |
| SpH | 27.85 | 31.59 | 31.74 |
| TrW | 25.53 | 33.19 | 32.70 |
| Constant | -4291.02 | -4504.24 | -4580.43 |
| | | | |

*For abbreviation see Table 1 and Figure 1.

The discriminant analysis as illustrated by the point of CV1 to CV2 mainly shows 2 clusters; 1 consists of specimens originally classified as *A. escheriana* and the other includes specimens which were classified as *A. thospitis* and *A. guttata*. The latter cluster seems to consist of 2 subclusters: 1 mainly consists of *A. guttata* and the other subcluster mainly includes *A. thospitis* (See Figure 4).

Table 5. Observed classification (rows) against predicted (columns).

| | Percent of | | | |
|---------------|----------------|---------------|------------|--------------|
| | Correct | | | |
| Species | Classification | A. escheriana | A. guttata | A. thospitis |
| | | | | |
| A. escheriana | 98 | 49 | 1 | 0 |
| A. guttata | 84 | 0 | 42 | 8 |
| A. thospitis | 84 | 0 | 8 | 42 |
| Total | 88.66 | 49 | 51 | 50 |
| | | | | |

Table 6. Canonical loadings in decreasing importance.*

| Variable | CV1 | Variable | CV2 | | | | |
|----------|-----------|----------|-----------|--|--|--|--|
| SpH | -0.493313 | ApW | 0.686959 | | | | |
| ShH | -0.480596 | TrW | -0.407456 | | | | |
| ShD | -0.346135 | ApH/ApW | -0.396973 | | | | |
| TrW | -0.311651 | SpH | -0.386501 | | | | |
| ApW | -0.275063 | ShD | 0.320868 | | | | |
| ApH | -0.201802 | ShD/ShH | 0.187056 | | | | |
| ShD/ShH | 0.187102 | ApH | 0.024679 | | | | |
| ApH/ApW | 0.051266 | ShH | -0.018809 | | | | |

*For abbreviation see Table 1 and Figure 1.

Figure 4 reveals that CV1 essentially serves to distinguish *A. escheriana* from *A. guttata* and *A. thospitis*, while *A. guttata* and *A. thospitis* are differentiated by CV2.

In the present study based on shell morphology, *A.* escheriana can be clearly differentiated from *A. thospitis* and *A. guttata* although the variability of the canonical scores of *A. escheriana* was greater than that of *A. thospitis* and *A. guttata*. On the other hand, the convergence of the canonical scores of *A. thospitis* and *A. guttata* support the idea that these two species may have a close evolutionary relationship (See Figure 4).

Group classification functions are used to determine to which group a new specimen most likely belongs. One

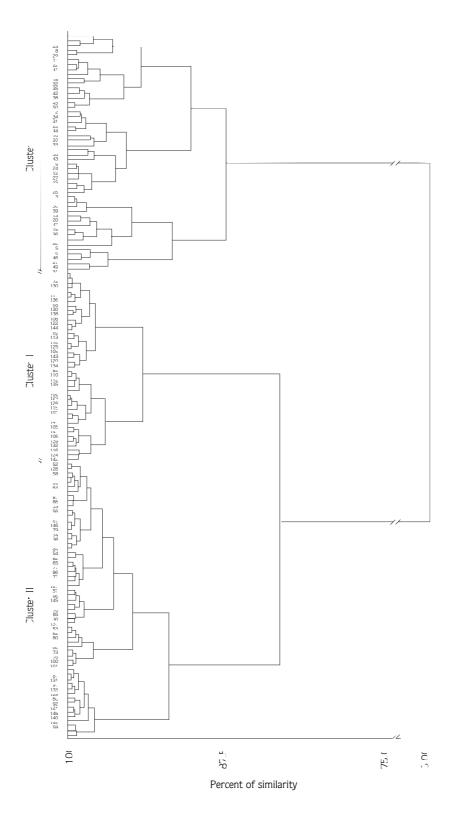


Figure 3. Dendrogram obtained by Ward's method

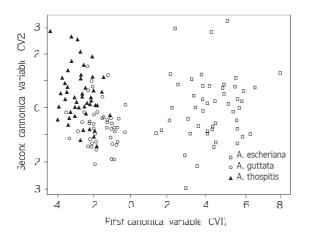


Figure 4. The scatter plot of CV1 by CV2

can apply these functions to new data and assign each case to the group with the largest function value for that case. These functions given in Table 4 were applied to the data matrix (Table 2) to see how these assignments work

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for our specimens. The results of this operation are given in Table 5. The largest percentage of specimens used in the present study were predicted correctly by group classification functions. We hope that the methods tried here on a limited number of individuals will be equally successful when further material is examined.

The order of importance of the characteristics (variables) for discriminating these species is shown in Table 6 in decreasing importance. SpH, ShH, ShD and TrW are most differentiating characters for *A. escheriana* from *A. thospitis* and *A. guttata*. The 4 most differentiating characters between *A. guttata* and *A. thospitis* are ApW, TrW, ApH / ApW and SpH in decreasing importance.

Consequently, the results of this study show that more precise identification can be made in the classification of *A. escheriana*, *A. thospitis* and *A. guttata* if the classical criteria are used together with the group classification functions.

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