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Original article (Orijinal araştırma)

A study on morphological variations of male *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) (Coleoptera: Helophoridae) in Türkiye[1]

Türkiye'deki erkek *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) (Coleoptera: Helophoridae)'un morfolojik varyasyonları üzerine bir araştırma

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Abstract

The geographic structure of Türkiye is well-suited for determining species diversity and interspecific variation as Türkiye has many regions with different altitudes and ecological characteristics. A large number of specimens belonging to the Helophoridae family belonging to the order Coleoptera were collected from different regions of Turkey between 2007-2021, examined and kept as museum material. Among the museum materials evaluated in terms of variation, *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) species were seen to be both more common and more diverse in each region. In this study, male specimens varying in color, size, pronotum, tarsi, and genital structure were selected among the samples collected from different regions and geographic locations within the same locality. A total of 17 different morphological variations were identified and listed by examining the pronotum, elytra, tarsi, and aedeagophore structures. The relationships of these variations with altitude have been evaluated. As a result of this study, it has been determined that variations of the same species can coexist in the same locality, as well as similar variations in localities in different geographical regions. In addition, it was determined that the size of the insects increased as the altitude increased.

Keywords: Altitude, Coleoptera, Helophoridae, *Helophorus aquaticus*, variation

Öz

Türkiye'nin coğrafi yapısı tür çeşitliliğini ve türler arası varyasyonu belirlemeye oldukça elverişlidir ve Türkiye'nin farklı rakımlara ve farklı ekolojik özelliklere sahip birçok bölgesi vardır. Coleoptera takımı Helophoridae familyasına ait çok sayıda örnek 2007-2021 yılları arasında Türkiye'nin farklı yörelerinden toplanmış, incelenmiş ve müze materyali olarak saklanmıştır. Varyasyon açısından değerlendirilen müze materyalleri arasında, *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) türlerinin her bölgede hem daha yaygın hem de daha çeşitli olduğu görülmüştür. Bu çalışmada, farklı bölgelerden ve farklı coğrafi konumlardan toplanan ve aynı lokasyonu paylaşan erkek örnekler arasından farklı renk, boyut, pronotum, tarsi ve genital yapılara sahip bireyler seçilmiştir. Pronotum, elytra, tarsi ve aedeagophore yapıları incelenerek toplam 17 farklı morfolojik varyasyon tanımlanmış ve listelenmiştir. Bu varyasyonların rakım ile ilişkileri değerlendirilmiştir. Bu çalışma sonucunda, aynı türün varyasyonlarının aynı lokalitede bir arada bulunabileceği gibi, farklı coğrafi bölgelerdeki lokalitelerde de benzer varyasyonların olabileceği tespit edilmiştir. Ayrıca rakım arttıkça böceklerin boyutlarının da arttığı tespit edilmiştir.

Anahtar sözcükler: Rakım, kınkanatlılar, Helophoridae, *Helophorus aquaticus*, varyasyon

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Introduction

Türkiye is located in the heart of a continent, called as the 'world island', which is known as one of the most diverse and resource-rich region extending over Euroasia and Africa mass land (Mackinder, 2004) This region function like a bridge connecting Türkiye to the Mediterranean, Black Sea, Balkans, Caucasus, and the Middle East Türkiye is a peninsula surrounded by seas on three sides, and differs in climates due to the variations in altitude ranging from 0-5137 m. Different ecological features and climates have also led to an increase in species diversity.

Taxa that are crowded with several species are older and have more diversity (McPeek & Brown, 2007). Diversification rate differences can result from both intrinsic factors (e.g., key innovations) and extrinsic factors (e.g., habitat shifts), or ecological limits (density-dependence) on clade diversity (Rabosky, 2009, 2010). It has been determined that the variations seen in insects are related to the environment (Daly, 1985; Palmer, 1994; Cepeda-Pizarro et al., 1996, 2003; Krasnov et al., 1996; Williams, 2001). There are many morphological characters (size of the hind limbs, structure of the elytra, form of the subelytral space, etc.) that are considered indicative of differentiation of populations (Lestrel, 1997; Møller & Zamora-Munoz, 1997; Shiokawa & Iwahashi, 2000; Bonacci et al., 2006; Garnier et al., 2006; Talarico et al., 2007).

Despite the morphological differences found in some Coleoptera families, there is a correlation between the size of their body parts. The body size in animals of the same species is an integral feature that affects both their physiology and behavior (Chown & Gaston, 2010). On the other hand, studies conducted in recent years have shown that changes in altitude also cause changes in the size of insects.

Size differences in widely distributed taxonomic clades have attracted attention of many scientists. Bergmann (1847), who defined this subject first, stated that larger populations and species were found in colder environments, while smaller ones were found in warmer environments. This theorem is called the “Bergmann rule”. A similar theorem, called “Allen's rule”, was used by Allen (1877) who argued that because animals living in cold climates must conserve as much heat as possible, they must have developed relatively low surface area-to-volume ratios to minimize the surface area from which heat radiates. He also noted in his study that the limbs of the species changed accordingly.

Although it is an established ecological principle, empirical support for Allen's rule is weak. Support for Allen's rule stems mainly from studies on a single species, and studies on different species have alternative adaptations that run counter to the predictions of the Bergmann and Allen rules (Nudds & Oswald, 2007).

There are literatures that address the relationships between body size and community structure for arthropods, specifically Coleoptera. While variability of body size is recorded in geographic gradients in many insect species, direction of the relationship may be different. Some species have an increase in size in the latitude direction, while we see a decrease in that of others (Blanckenhorn & Demont, 2004). In the latitudinal slope, length of light period, air temperature and duration of the growing season tend to decrease due to regional, climatic and seasonal conditions. In particular, the temperature changes constantly depending on latitude and altitude.

In a study on *Teleogryllus emma* (Ohmachi & Matsuura, 1951) (Orthoptera: Gryllidae) populations, it was observed that the body size of the Emma field cricket, represented by head width, tended to be smaller from south to north and from low to high, reaching larger sizes in warm regions than in cold regions (Masaki, 1967). In another study on *Carabus dehaanii* subsp. *tosanus* (Nakane & Iga&Ueno, 1953) (Coleoptera: Carabidae) in Japan, it was determined that the body lengths of insects grown at high temperatures in the setup in the laboratory were greater than those grown at low temperature (Tsuchiya et al., 2012).

Although there are many studies parallel to the Bergman & Allen rules, there are many exceptions to both rules. Body size at the intraspecific level may increase, decrease, or not change significantly towards higher latitudes (Blanckenhorn & Demont, 2004; Shelomi, 2012). In addition, clinical body size variation in arthropods generally follows the opposite of the Bergmann's rule (Mousseau, 1997).

In another study conducted with the carabid beetles, it was determined that the sizes of these insects showed an adaptation contrary to the Bergmann's rule (Park, 1949). On the Japanese islands, the elytra length of *Phyllotreta striolata* (Fabricius, 1803) (Coleoptera: Chrysomelidae) has again been found to contradict this rule (Hukushima, 1960). The length and width of the elytra, pronotum and head were measured in the selected specimens of *Pterostichus montanus* (Motschulsky, 1844) (Coleoptera, Carabidae). Based on the results obtained, it was noted that the species living in the coastal region were the smallest, and the species living in the low mountains were the largest (Sukhodolskaya et al., 2021).

Perhaps many other examples can be found that contradict Bergmann's rule. For some species, it is possible that there are different factors other than temperature and latitude that can affect the dimensions. In a study with carabids, the larger *Pterostichus* and *Carabus* species are known to prefer less disturbed habitats (Blake et al., 1994). In addition, it has been determined that there is a relationship between body size and development time, and that development time causes variation in body size (Roff, 1980). In addition, it has been reported that availability of food, especially during the growth period, has an effect on size (Yom-Tov & Geffen, 2011; Brandmayr & Pizzolotto, 2016). The morphometric analyses (Novotny & Wison, 1997) performed on species which contain the Auchenorrhyncha group including foam beetles, revealed that there is a significant relationship between body size and Hemipter, which uses phloem and xylem sap as food. Larger beetles have also been reported to be more resistant to famine, drought and temperature extremes and winter (Kingsolver & Huey, 2008).

The colors of insects and the variations in these colors are quite remarkable. It has been observed that coloration of small insects is related to the ambient temperature, and the heat absorption in these animals can be affected by their coloration, since dark colored forms absorb solar radiation faster. In this respect, dark forms in many polymorphic species tend to be seen at a higher frequency in cold habitats than in open habitats that are directly exposed to the sun. Melanism due to thermal selection has been shown to be an adaptive value for Colias butterflies in the alpine cold habitats. Thermal melanism has been discussed as an important component of evolutionary adaptation in many species such as foam beetles, grasshoppers, and spiders (Yurtsever, et al., 2005). However, in the study of *Hologymnetis argenteola* (Bates, 1889) and *Hologymnetis cinerea* (Gory & Percheron, 1833) (Coleoptera: Scarabaeidae: Cetoniinae) species, the dorsal color and degree of punctuation of insects varied significantly with no apparent relationship to geography or altitude (Ratcliffe & Deloya, 1992).

In this 15-year-long study, it was determined that the Helophoridae family was denser than other species among the aquatic insects collected from different cities and different altitudes of Türkiye. We have determined that this family has a high adhesion ability at different altitudes. Among the analyzed samples, it was observed that *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) showed both morphological variation and different sizes.

The current study discusses the effects of altitude on size and variations of *H. aquaticus* obtained from different regions of Türkiye and observed in localities with different ecological characteristics between 2007 and 2021.

Colors, body lengths, aedeagophore structures, and legs of the samples were photographed and length measurements were recorded. Comparisons of the measured parameters were evaluated according to the altitude at which the samples were collected. It was aimed to investigate whether the altitude affects color, height, and aedeagophore lengths. The results were evaluated separately for each parameter.

Materials and Methods

Helophorus aquaticus selected for the research has a very wide distribution in the world. They spread from sea level to the peaks of high mountains (Angus, 1988). They are usually found in stagnant fresh waters, small, shallow and muddy ponds, temporary puddles, shallow parts of standing waters and streams, and prefer shady waters on swampy ground. Normally in spring, they lay cocoons, which contain about 15 eggs, into the mud at the water's edge. The larvae do not emerge until the next spring (or early summer). Larval development takes about three weeks. Adults can usually be seen from spring to autumn (Hansen, 1987). It is known that they live in almost every region of Turkey. It has records from approximately 40 provinces (Polat et al., 2021).

This study was conducted to compare in terms of variation the male *H. aquacus* species belonging to the Helophoridae family collected from various regions of Turkey within 15 years and to examine whether altitude causes morphological changes in individuals belonging to the same species. The localities are lettered alphabetically, and the number of individuals, their coordinates, annual averages of temperature and altitudes of the provinces where they are gathered are presented in Table 1. The map of Turkey where the localities are marked is given in Figure 1.

The samples were collected from the shore of marshes, geological formations and lakes, through slow-flowing water, grassy areas where aquatic insects can live, and places where vegetative decay is high. Collected samples were taken to the laboratory after treatment with ethyl acetate.

Table.1. Localities where samples were collected

Sample	Number of samples	Locality	Annual average temperature (°C)	Altitude (m)
a	3♂♂	Ordu, Ünye: Çatalpınar, 41°06'06K 37°14'06D, 28.V.2007	14.5	41
b	3♂♂	Amasya, Merkez: Doğantepe, 40°34’37K 35°36’43D, 03.VI.2008	13.6	491
c	4♂♂	Tokat, Erbaa: İverönü, 40°36’30K 36°36’03D, 29.V.2007	12.5	531
d	4♂♂	Tokat, Yeşilyurt: 40°00’30K 36°11’59D, 01.VI.2007	12.5	1065
e	4♂♂	Amasya, Hamamözü: Yeniköy, 40°48’01K 35°09’00D, 03.VI.2008	13.6	1081
f	4♂♂	Kayseri, Develi: Karapınar, 38°24’11K 35°19’29D, 30.V.2009	10.7	1084
g	3♂♂	Kahramanmaraş, Elbistan: Kuşkayası, 38°18'22K 37°05'48D, 09.IX.2011	16.7	1155
h	4♂♂	Tokat, Zile: Yünlü, 40°23’14K 35°51’48D, 03.VI.2008	14.5	1212
i	4♂♂	Giresun, Dereli: Bektaş plateau, 40°37'08K 38°16'36D, 24.V.2007	14.6	1354
j	4♂♂	Kahramanmaraş, Göksun: Soğukpınar, 38°03'15K 36°34'37D, 26.VI.2012	16.7	1361
k	3♂♂	Kayseri, Yahyalı: Avlağı, 38°00’05K 35°32’41D, 25.VI.2010	10.7	1483
l	3♂♂	Kayseri, Pınarbaşı: 38°43’11K 36°23’00D, 25.VI.2011	10.7	1507
m	5♂♂	Erzurum, Erzurum Marshes 40°01'09"K 41°18'52"D, 1756m, 19.06.2017	5.7	1756
n	3♂♂	Van, Özalp: Çalımlı, 38°39'50K 43°47'55D, 1909m 2021	9.4	1909
o	3♂♂	Kayseri, Hisarcık: Erciyes Mountain 38°35’26K 35°30’18D, 25.V.2010	10.7	1972
p	4♂♂	Muş, Varto:Hamurpet Lake, 39°08'03"K 41°42'14"D, 21.06.2017	9.8	2169
q	3♂♂	Erzurum, Hınıs: Erzurum Geological formations, 39°24'04”K 41°26'08”D, 01.07.2017	5.7	2661

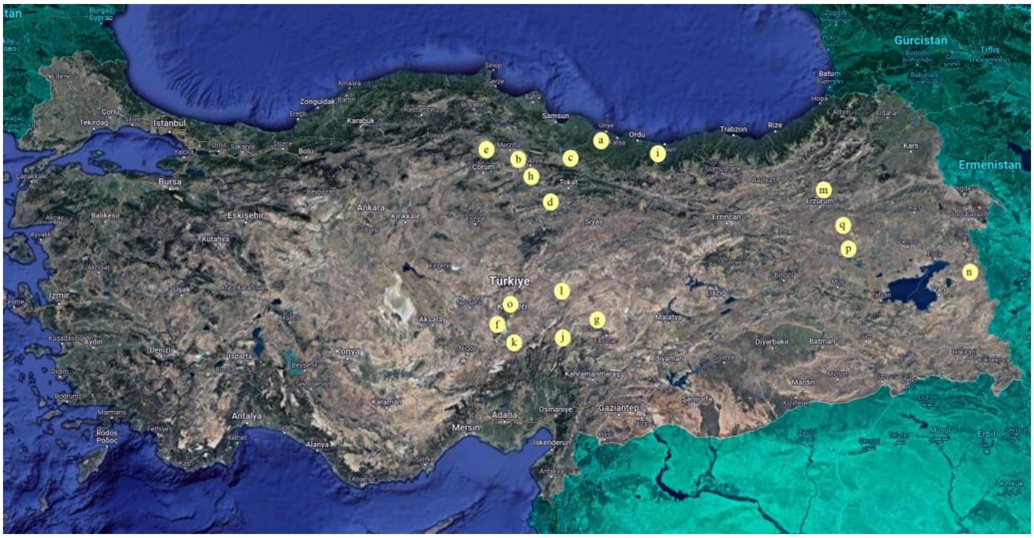

Figure 1. Türkiye map with localities where samples were collected.

Species were identified by examining the morphological characters of the samples brought to the laboratory. Male *H. aquaticus* species belonging to the Helophoridae family were selected among the identified species to determine their morphological variations.

Aedeagophores were soaked in 10% KOH solution for 1-2 hours to separate the muscle tissue around the chitin structure. They were then placed on a slide with a drop of glycerin. Measurements were made by drawing aedeagophore shapes on Nikon SMZ1500 stereomicroscope. The common and distinctive features of the species were photographed with the Leica DFC295 brand macroscope. +5 light setting and 40% iris setting were used in all photo shoots. Color samples of body parts were taken from photographs for each species using the 31x31 average scale in Adobe Photoshop CS6 program and color determinations were made by comparing them with the Pantone color library.

Body measurements were taken using a micrometric eyepiece. Body lengths and widths were recorded by measuring the longest and widest parts of the body and regional coloration conditions in the body were examined. The structures and dimensions of the pronotum were compared. The coloration of the legs and their dimensions compared to the body were evaluated. Paramere structures and dimensional differences were evaluated by comparing aedeagophore structures.

Statistical analysis

Statistical analyses of the study were performed with SPSS 20.0 (IBM Inc, Chicago, IL, USA) program. Descriptive statistics were presented as numerical variables using the mean and standard deviation (mean±SD) and categorical data as frequency (percentage ratio). ANOVA was used to compare morphometric measurements according to the types of samples. Tukey HSD post-hoc test was preferred for pairwise comparisons of the results. The correlation values between the altitude of the species and all morphometric measurements were calculated by Pearson's correlation analysis. Simple linear regression models were established to determine the effect levels of elevation on the measurements. A $p<0.05$ value was considered statistically significant for the type-I error rate of 5% in the analyses.

Results and Discussion

The morphological variations of the samples were examined by comparing the data obtained after the analysis of the morphometric characters Detected morphological variations are presented under the headings. A total of 62 samples collected from 17 different elevations were included in the study. 3 or 4 samples were collected foreach elevation. Descriptive information about the collected samples are presented in Table 1. In the correlational analysis, most of the measurements and altitude were found to have a positive and significant relationship. Only Elytra width and leg height were not associated with elevation. The highest correlation values with the elevations of the samples were found between body height (r=0.880; p<0.001) and pronotum width (r=0.822; p<0.001) (Table 5). Morfometric measurements of all samples were compared. It was observed that all the values differed significantly between samples (p<0.001). Measurements did not differ significantly between species collected from the same locality, but significant differences were observed between species collected from different locations for each morphometric measurement (Tables 3 & 4).

Since there were significant relationships between altitude and other measurements, simple linear regression models were created to determine the effect of altitude on the measurements and to create a prediction model. Significance and explanatory coefficient information of the models are presented in the table (Table 6). In parallel with the correlation values, the best models explaining the elevation were trunk height, pronotum height and pronotum width. Regression models including elytra width and leg length as predictors were not significant.

Color

In our study, samples collected in different years and preserved as museum material were used. While transforming the samples into museum material, no chemicals were used that would cause color change or impair pigmentation.

Our samples were examined using the Nikon SMZ1500 stereomicroscope. It has been observed that the colors of individuals collected from the same locality are very close to each other. For this reason, the most intact body parts were selected among the samples for photographing. Selected specimens were photographed using a Leica DFC295 macroscope at +5 light setting and 40% iris setting. Photographs of the samples were uploaded to Adobe Photoshop CS6 program one by one; color samples were taken from the body parts with an average scale of 31x31 and compared with the colors in the Pantone color library.

The head is generally dark brown to black. The golden yellow and black tubercles are scattered all over the surface. Antennas are brown, maxillary palps are dark brown (Figure 2). Pronotum can be black or dark brown. Sternite appears dark brown or black (Figure 3). Elytra is brown and pale spots are sparsely distributed throughout the elite. Elytral speckles and a dark speck of "Λ" are evident (Figure 2). When viewed from the ventral; the last segment of the tarsi and the nails are dark black in color, while the other parts range from light brown to dark brown (Figure 5).

Among our samples, head, pronotum, elytra, and leg colors were examined. It was determined that the colors of the head, pronotum, and elytra generally varied between brown and black. On the legs, when viewed from the ventral side, the last segment of the tarsi and the claws were dark black, and the other parts were light brown to dark brown.

Although a study done by Ratcliffe & Deloya (1992) stated that the coloration was not related to geography or altitude, it was observed in this research that the colors of our samples were darker depending on the increase in altitude. Considering that the temperature decreases at higher altitudes, the idea that insects need to be darker in color to better cool the solar radiation seems more likely. Similarly, Yurtsever et al. (2005) emphasized that those living in cold habitats are darker in many polymorphic species. However, this may show different results for different groups.

The colors of the body parts of the samples observed at different altitudes are given in the Table 2. It is noteworthy that the samples collected from higher altitudes were darker in color.

Figure 2. Dorsal views of the samples.

Table 2. The colors of the body parts at different altitudes

Sample	Altitude (m)	Head			Pronotum			Elytra		
		Black	Dark brown	Brown	Black	Dark brown	Brown	Black	Dark brown	Brown
a	41	X			X			X		
b	491		X				X			X
c	531			X			X		X	
d	1065		X				X			X
e	1081	X			X				X	
f	1084			X			X			X
g	1155	X					X			X
h	1212	X			X			X		
i	1354	X			X				X	
j	1361	X				X			X	
k	1483	X				X			X	
l	1507		X				X			X
m	1756	X				X			X	
n	1909	X			X			X		
o	1972		X			X			X	
p	2169	X			X			X		
q	2661		X			X			X	

The sizes of the body and body parts

The sizes of the body

The measurements of the body and body parts of our samples were made morphometrically. The full length and width ratios of the body, the length and width of the pronotum and elytra, and the lengths of the legs and aedeagophore parts were recorded. Measurements were made from the longest and widest parts of the body or body parts. The result of these measurements is presented in the Tables 3 & 4. Scatter plots and regression lines were drawn for each parameter (Figure 6).

The data presented in Tables 3&4 show that there are differences between the groups. The letters next to each measured parameter indicate this difference. The letters given to the examples were chosen according to the altitude. The sample collected from the lowest altitude is given the letter “a” and the sample collected from the highest altitude is given the letter “q”. Thus, as a result of the test, the letter "a" was assigned to the largest sized sample, and the letter "q" to the smallest sized sample. When the results are examined, it is seen that there is an altitude/size relationship in all parameters, but the largest species are found at the highest altitude and the smallest species at the lowest altitude.

Table 3. The size of body parts depending on altitude (The letters next to each measured parameter indicate the difference)

Sample	Altitude (m)	Body height (mm)	Body width (mm)	Elytra height (mm)	Elytra width (mm)	Pronotum height (mm)	Pronotum width (mm)
a	41	4.46±0.049 j	2.20±0.018 ab	3.66±0.036 bc	2.16±0.019 efg	0.88±0.007 h	1.55±0.012 j
b	491	4.53±0.075 ij	1.98±0.008 bc	3.37±0.047 c	2.02±0.013 hi	0.89±0.009 h	1.45±0.017 l
c	531	4.69±0.049 j	1.95±0.024 bc	3.11±0.027 c	1.94±0.009 i	0.81±0.010 j	1.46±0.009 l
d	1065	4.66±0.061 j	1.98±0.023 bc	3.24±0.045 c	1.96±0.017 i	0.92±0.011 g	1.49±0.014 k
e	1081	5.11±0.080 fgh	2.15±0.035 abc	2.13±0.022 d	3.39±0.040 a	0.95±0.011 f	1.69±0.010 d
f	1084	5.10±0.043 fgh	2.19±0.022 ab	3.45±0.044 c	2.23±0.025 def	0.92±0.009 g	1.63±0.025 h
g	1155	5.27±0.008 cdef	2.32±0.023 c	3.76±0.024 bc	2.36±0.020 c	0.98±0.010 de	1.69±0.021 d
h	1212	5.30±0.108 cde	2.10±0.094 abc	3.40±0.055 c	2.14±0.112 fg	0.99±0.051 d	1.70±0.055 d
i	1354	5.05±0.051 gh	2.17±0.018 abc	3.50±0.036 c	2.20±0.011 efg	0.84±0.008 i	1.59±0.015 i
j	1361	5.14±0.028 efgh	2.12±0.028 abc	3.49±0.036 c	2.19±0.018 efg	0.92±0.010 g	1.65±0.011 gh
k	1483	5.35±0.041 cd	2.09±0.024 abc	3.42±0.011 c	2.13±0.029 fg	0.99±0.008 d	1.68±0.020 de
l	1507	5.19±0.034 defg	2.08±0.002 abc	3.42±0.016 c	2.05±0.022 ef	0.97±0.008 e	1.66±0.020 fg
m	1756	5.00±0.069 h	2.09±0.075 abc	3.26±0.128 c	2.08±0.051 gh	0.94±0.076 f	1.67±0.063 ef
n	1909	5.42±0.079 c	2.15±0.017 abc	3.55±0.019 c	2.16±0.020 efg	0.95±0.010 f	1.69±0.017 d
o	1972	5.73±0.044 b	2.31±0.037 ab	3.60±0.022 a	2.27±0.022 cde	1.03±0.011 c	1.75±0.022 c
p	2169	5.74±0.038 b	2.31±0.037 ab	3.77±0.028 bc	2.33±0.016 cd	1.08±0.011 b	1.85±0.012 b
q	2661	6.48±0.061 a	2.61±0.020 a	4.35±0.044 ab	2.59±0.013 b	1.21±0.008 a	1.92±0.029 a
	Sign.	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**

* Significant at 0.05 level according to ANOVA test, the pairwise comparison was performed by Tukey HSD, Sign.: significant level p.

Many studies reporting the presence of large species in high altitudes suggest that various reasons may cause this situation. Blake et al. 1994 attributed this to the disturbance of habitats, Novotny & Wison (1997), Yom-Tov & Geffen (2011) and Brandmayr & Pizzolotto (2016) attributed this to an increase in the amount of nutrients, and Roff (1980) to an increase in development time due to a decrease in temperature. Kingsolver & Huey (2008) stated that larger insects are more resistant to famine, drought and extreme temperatures and winter. All these assumptions are likely to be true.

Table 4. The size of aedeagophore parts and leg height depending on altitude (The letters next to each measured parameter indicate the difference)

Sample	Altitude (m)	Basal-aedeagophore tip (mm)	Basal-paramere tip (mm)	Basal part (mm)	Top width (mm)	Leg height (mm)
a	41	0.76±0.008 fg	0.79±0.005 ef	0.23±0.001 def	0.23±0.001 cdef	2.09±0.014 g
b	491	0.72±0.009 i	0.77±0.008 gh	0.24±0.002 def	0.24±0.002 fg	2.01±0.020 i
c	531	0.84±0.011 c	0.88±0.004 bc	0.25±0.002 d	0.25±0.002 bc	2.25±0.024 b
d	1065	0.79±0.008 e	0.78±0.002 ef	0.25±0.002 cd	0.25±0.002 a	2.25±0.027 b
e	1081	0.73±0.008 i	0.76±0.008 h	0.25±0.002 cd	0.25±0.002 defg	1.95±0.022 k
f	1084	0.74±0.008 h	0.80±0.004 de	0.23±0.004 ef	0.23±0.004 fg	2.22±0.025 c
g	1155	0.77±0.008 ef	0.79±0.003 def	0.24±0.003 de	0.24±0.003 fg	2.18±0.025 e
h	1212	0.75±0.009 gh	0.75±0.087 fg	0.25±0.077 d	0.25±0.077 ab	1.99±0.105 j
i	1354	0.87±0.011 b	0.87±0.012 bc	0.24±0.002 de	0.24±0.002 efg	2.25±0.024 b
j	1361	0.76±0.004 fg	0.80±0.007 def	0.22±0.002 f	0.22±0.002 g	1.89±0.014 l
k	1483	0.77±0.006 ef	0.79±0.007 def	0.23±0.002 def	0.23±0.002 cde	2.22±0.028 c
l	1507	0.77±0.003 ef	0.81±0.010 d	0.24±0.001 de	0.24±0.001 efg	2.01±0.023 i
m	1756	0.78±0.006 ef	0.81±0.007 d	0.25±0.088 cd	0.25±0.088 cdef	2.28±0.068 a
n	1909	0.76±0.010 fgh	0.80±0.009 de	0.24±0.002 def	0.24±0.002 defg	2.20±0.016 d
o	1972	0.84±0.003 c	0.87±0.007 bc	0.27±0.002 bc	0.27±0.002 ab	2.06±0.014 h
p	2169	0.94±0.008 a	0.96±0.012 a	0.28±0.003 ab	0.28±0.003 ab	2.21±0.024 cd
q	2661	0.81±0.008 d	0.89±0.006 b	0.29±0.002 a	0.29±0.002 cd	2.15±0.033 f
	Sign.	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**

* Significant at 0.05 level according to ANOVA test, the pairwise comparison was performed by Tukey HSD, Sign.: significant level p.

Pronotum

As seen in the Table 3 pronotum is 0.8-1.2 mm long and 1.4-1.9 mm wide. It can be black or dark brown. The tubercles are smaller than those in the head and are spread over the whole area. Sternite appears dark brown or black. The part between the cocca and the head is short, straight looking, and slightly raised. Variations were also detected in the pronotum of our samples. Some have greater depths of grooves (g, h, p, q). In some of the samples, the margins of the pronotum are sharper (f, l, p) (Figure 3).

Head and pronotum are frequently used in body measurement comparisons, especially in insects. In our study, the most significant results were obtained from the pronotum measurements. ANOVA showed that pronotum width and length were significantly affected by altitude. ANOVA data showing that *H. aquaticus* species have larger and wider pronotum at high altitudes and making the differences according to the lettering given to the samples are presented in Table 3.

Aedeagophore

Aedeagophore of samples are 7.2-9.6 mm long and the base piece is longer than the parameres. Parameres approach each other towards the tip. The middle lobe is shorter and thicker than parameres. The pedestal arms are curved and their loose ends converge (Figure 4).

In some of our samples, the parameres are wider and end by tapering (a and n), some of them have slight indentations on the paramere edges (e and m), in some, the struts are more curved (d, p, and o), in some, the struts are shorter and it ends at the tip of the basal part (d, h and m), in some, the basal part ends with sharper lines (c, f, and k) (Figure 4).

While making aedeagophore measurements, 4 parameters (Basal-aedeagophore type, Basal-paramere type, Basal part, Top width) were taken as basis. When the obtained values were compared with the altitude, it was seen that the 4 parameters increased in direct proportion to the altitude. ANOVA showed that each parameter was significant and reliable (Table 4).

Figure 3. Pronotum views of samples.

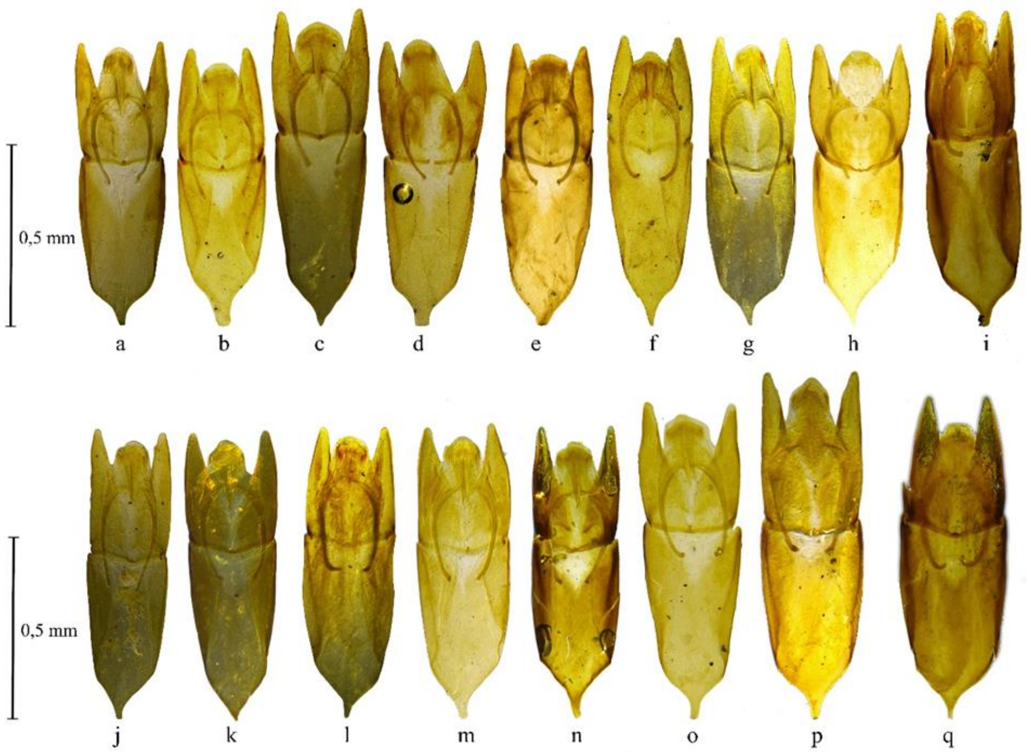

Figure 4. Aedeagophore views of samples.

Leg

From the ventral view, the legs are thick and long. The last segment of the tarsi and the nails are dark black in color, while the color of other parts range from light brown to dark brown. The denticles on the last part of the seventh abdominal sternites are small. In some of the samples, the legs are yellowish-brown (g, k, m, q), some are blackish (f, j), others are dark brown (Figure 5).

The leg lengths of each sample were measured and its relationship with height was tried to be determined. However, the results were not found to be significant since no parallelism could be found with the correlation values.

Figure 5. Legs views of samples.

Table 5. The correlation between altitude and other morphological measurements

Altitude (m)	Body height (mm)		Body width (mm)		Elytra height (mm)		Elytra width (mm)		Pronotum height (mm)		Pronotum width (mm)	
r; *p*	0.880	<0.001*	0.616	<0.001*	0.415	0.001*	0.134	0.300	0.756	<0.001*	0.822	<0.001*
	Basal-aedeagophore tip (mm)		Basal-paramere tip (mm)		Basal part (mm)		Top width (mm)		Leg height (mm)			
r; *p*	0.380	0.002*	0.400	0.001*	0.325	0.010*	0.325	0.010*	0.161	0.210		

**: Significant at 0.05 level according to Pearson Correlation Analysis; r: correlation coefficient; p: significant level.*

Table 6. The regression models of morphological measurements on altitude

	R^2	F	*p*	Model
Body height (mm)	0.774	205.016	*<0.001**	4.271+0.001*altitude
Body width (mm)	0.379	36.658	*<0.001*	1.948+0.001*altitude
Elytra height (mm)	0.172	12.492	*<0.001*	2.967+0.001*altitude
Elytra width (mm)	0.018	1.095	*0.300*	Not significant
Pronotum height (mm)	0.571	79.923	*<0.001**	0.8+0.001*altitude
Pronotum width (mm)	0.675	124.70	*<0.001**	1.43+0.001*altitude
Basal-aedeagophore tip (mm)	0.144	10.127	*0.002**	0.742+3.5E-05*altitude
Basal-paramere tip (mm)	0160	11.449	*0.001**	0.755+4.39E-05*altitude
Basal part (mm)	0.105	7.075	*0.010**	0.222+1.8E-05*altitude
Top width (mm)	0.105	7.075	*0.010**	0.222+1.8E-05*altitude
Leg height (mm)	0.026	1.605	*0.210*	Not significant

**: Significant at 0.05 level according to Linear Regression, R^2: Coefficient of determination*

In the present study, the morphological examination of the specimens belonging to the Helophoridae family living in Türkiye was conducted. Among the samples examined, species of *H. aquaticus* that showed variation among the collected samples from the same locality were selected, the differences were evaluated morphometrically. The morphological differences of the species were stated by making comparisons among themselves. Seventeen morphological variations have been identified in terms of color, size, and structure (aedeagophore, pronotum, elytra, and leg). Differences were noted. It is believed that the results of this study will now enable accurate and faster identification of *H. aquaticus* species. In addition, by giving the heights of the locations where the samples were collected, the relationship between morphological differences and altitude was determined.

It has been determined that there are serious relationships between altitude and size in many insect species. In some, the size increased with altitude, while in others it decreased. In this study, 11 parameters (body height, body width, elytra height, elytra width, pronotum height, pronotum width, basal-aedeagophore type, basal-paramere type, basal part, top width, leg height) were measured in order to understand the effect of altitude. The ANOVA analysis performed in the study using all these parameters indicated a statistically significant effect of altitude on size, at $p<0.05$. Specifically, all the data show that small specimens of *H. aquaticus* live at low altitudes, while large specimens live at higher altitudes. Our results contradict the Bergmann and Allen rules. being in line with many other studies in literature (Park, 1949; Hukushima, 1960; Mousseau, 1997; Novotny & Wison, 1997; Blanckenhorn & Demont, 2004; Shelomi, 2012; Sukhodolskaya et al., 2021). With this study, it has been shown that the Bergmann and Allen rules are not inclusive for all species. In addition, this study confirmed that the variations are directly related to the environment.

Statistical analyses performed in this study show that the size of all body parts of our samples increased with height, except for elytra width and leg length. In parallel with the correlation values, the best models explaining the elevation were trunk height, pronotum height and pronotum width. Of course, it may not be possible for these results to be a common rule for all species. However, it has been observed that the relationship between body and elytra lengths of *H. aquaticus* and altitude results in the opposite direction of Bergman and Allen's rules, so that both rules cannot be generalized to all species. There may be many factors affecting the size of coleopters, but this study has revealed that one of them is altitude.

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