Original article (Orijinal araştırma)

A chemotaxonomic approach to fatty acid composition of the genera *Helochares* Mulsant, 1844 and *Coelostoma* Brullé, 1835 (Coleoptera: Hydrophilidae)[1]

Helochares Mulsant, 1844 ve *Coelostoma* Brullé, 1835 (Coleoptera: Hydrophilidae) cinslerinin yağ asiti kompozisyonuna taksonomik bir yaklaşım

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Abstract

In this study, total lipid and fatty acid composition of insects belonging to the genera *Helochares* Mulsant, 1844 and *Coelostoma* Brullé, 1835 (*Coelostoma orbiculare* Fabricius, 1775; *Coelostoma transcaspicum* Reitter, 1906; *Helochares obscurus* Müller, 1776; *Helochares lividus* Forster, 1771) of Hydrophilidae (Coleoptera) family collected from Bingöl Province were determined. Specimens were collected in 2015. Fatty acid component of the insects was determined by mass-gas chromatography (GC-MS). Total saturated fatty acids (ΣSFA) were between 23.9-40.8%, total monounsaturated fatty acids (ΣMUFA) were 21.6-53.2% and total polyunsaturated fatty acids (ΣPUFA) were 14.3-27.4%. Myristic acid (14:0), pentadecanoic acid (15:0) palmitic acid (16:0), heptadecanoic acid (17:0) from SFA, palmitoleic acid (16:1n-7), oleic acid (18:1n-9) from MUFA; linoleic acid (18:2n-6), linolenic acid (18:3n-3), arachidonic acid (ARA, 20: 4n-6), eicosapentaenoic acid (EPA, 20: 5n-3) were the most important fatty acids. ANOSIM results showed that only the difference among the species was significant (R=0.63); the difference among subfamilies (R=0.17) and among the genera (R=0.17) were partially significant and that the difference among the families (R=0.08) was not significant.

Keywords: Bingöl, chemotaxonomic approach, *Coelostoma,* fatty acids, *Helochares*

Öz

Bu çalışmada, Bingöl İli'nden toplanan Hydrophilidae (Coleoptera) familyasının *Helochares* Mulsant, 1844 ve *Coelostoma* Brullé, 1835 cinsine (*Coelostoma orbiculare* Fabricius, 1775; *Coelostoma transcaspicum* Reitter, 1906; *Helochares obscurus* Müller, 1776; *Helochares lividus* Forster, 1771) ait böceklerin toplam lipit ve yağ asiti kompozisyonu belirlenmiştir. Örnekler 2015 yılında toplanmıştır. Böceklerin yağ asiti bileşeni gaz kromatografisi (GC-MS) ile belirlenmiştir. Toplam doymuş yağ asitleri (ΣSFA) %23.9-40.8, toplam tekli doymamış yağ asitleri (ΣMUFA) %21.6-53.2 ve toplam çoklu doymamış yağ asitleri (ΣPUFA) %14.3-27.4 arasında değişim göstermiştir. SFA' dan miristik asit (14: 0), pentadekanoik asit (15: 0) palmitik asit (16: 0), heptadekanoik asit (17: 0); MUFA'dan palmitoleik asit (16: 1n-7), oleik asit (18: 1n-9); ve PUFA' dan linoleik asit (18: 2n-6), linolenik asit (18: 3n-3), araşidonik asit (ARA, 20: 4n-6), eikosapentaenoik asit (EPA, 20: 5n-3) en önemli yağ asitleri olarak saptanmıştır. Hydrophilidae familyası bireylerinde sadece türler arasındaki farkın nispeten önemli olduğu (R=0.63), alt familyalar (R=0.17) ve cinsler (R=0.17) arasındaki farkın kısmen önemli olduğu ve familyalar (R=0.08) arasındaki farkın önemli olmadığı ANOSIM sonuçlarıyla ortaya konulmuştur.

Anahtar sözcükler: Bingöl, kemotaksonomik yaklaşım, *Coelostoma,* yağ asitleri, *Helochares*

Introduction

The great majority of Hyrdophilidae are aquatic, but there are also species living in semiaquatic and terrestrial habitats. Although they are found in many different habitats, many members of the Hydrophilidae family live among the plants and mosses in the shallow parts of rivers, lakes, ponds, canals, pools, trenches, streams and temporary water deposits (Hansen, 1987). It is known that the semiaquatic members are found in soil close to a water source or under rubble, such as plants and straws, which start to rot, whereas terrestrial members are known to be found inside or under feces of vertebrate animals such as goats and cows, in places where there is a lot of vegetative decay, and even in bird nests (Cercyon) (Fikáček, 2006). They are caught with instruments such as fine sieves and light traps, and they are generally black, brown or yellowish color (Angus, 1992). Adults generally feed with watery plants, mosses and vegetative detritus, and rarely consume food of animal origin. Fish and water birds love eating them. The larvae are carnivorous (Demirsoy, 1997). Three genera and 58 species of Hydrophilidae have been recorded (Gentili, 2000; Gentili & Whitehead, 2000; İncekara et al., 2005; Darılmaz & Kıyak, 2006a, b; Kıyak et al., 2006) and 13 new species reported by Mart (2009) in Turkey.

Lipids important in the human and animal diets are a group of compounds consisting of glycerol and fatty acids (Gilby, 1965). Fatty acids are fairly important in that they are the main component of fats, and they also participate in the structure of the cell membrane and are the precursors of bioactive metabolites. Fatty acids stored in the form of triacylglycerol are vital for insects as they serve as the main energy source in times when they do not find food and during long flights (Downer & Matthews, 1976; Beenakkers et al., 1985).

Some of the fat reserves of insects are met by assimilation of vegetable oils and partly by synthesis from carbohydrates (Stanley-Samuelson et al., 1988). Lipids are important in insect biochemistry as energy sources, hormones and structural compounds. Lipids have also been identified as the major source of energy in insect embryogenesis (Gilbert, 1967). As an energy source, nutritional lipids are more important than nutritional proteins. Fatty acids, which have important functions in all organisms, such as energy storage, transport, mobilization, and being structural components of bio membranes, have a few specific functions in insects. Fatty acids are also important as precursors in the biosynthesis of waxes, pheromones and eicosanoids. However, it is also known that they components of protection secretions (Wakayama et al., 1980; Stanley-Samuelson et al., 1988; Başhan, 1996).

The first known physiological role of eicosanoids has been discovered in insects (Stanley, 2000). Eicosanoids are an important intermediate substance in the biology of invertebrates in many areas such as production, ion transport (Stanley, 2000), hormone signal transduction system (Keeley et al., 1996) and immune system (Stanley, 2000). Eicosanoids also have a function in animal populations where there is a hunter-prey relationship or a host-parasite relationship (Stanley, 2000). Insects can synthesize saturated and monounsaturated fatty acids, like highly structured animals, through similar synthesis processes (Thompson, 1979). In addition, biological factors such as age and gender of the insects, temperature, adult nutrition and duration of activity affect fatty acid composition (Cohen, 1990). Canavoso et al. (2001) suggested that lipid and fatty acid contents vary depending on the species, gender and stage of development, and therefore fatty acid profile should be examined with respect to gender and developmental stages. We used insect groups living both in terrestrial and aquatic environments. In this study, intergenus and interspecies total lipid, and within and between species fatty acid compositions of Hydrophilidae (Coleoptera) family (*Helochares* and *Coelostoma*) collected from Bingöl Province were determined. In addition, PRIMER-version-7 statistical software was used to demonstrate chemotaxonomic similarities.

Materials and Methods

This study was conducted in two stages, namely field and laboratory studies.

Field studies

Materials used in this study were collected from Bingöl Province central, districts and various villages within these districts. Samples were collected between May and June 2015 by sifting between plants and moss in the shallow sections of various streams, springs, creeks, sediments and hot water sources. The species investigated were *Helochares lividus* (Forster, 1771)*, Helochares obscurus* (Müller, 1776)*, Coelostoma orbiculare* (Fabricius, 1775) and *Coelostoma transcaspicum* Reitter, 1906 because only two the species could be identified in the research area. Identification of the species was made according Mart (2005). Mouth aspirator, stereomicroscope, numbered insect needles and glassware were used to collect these species. Collected insects were brought to the laboratory in storage jars without being killed. No chemicals were used during the collection of samples because samples used in fatty acid analysis. The samples brought to the laboratory were stored in the freezer at -80°C for use in studies after the species were diagnosed.

Material examined. *Helochares lividus,* Bingöl (central), Ağaçeli Village, 28.VI.2015, 14 ♂♂, 16 ♀♀; *H. obscurus,* Bingöl, Solhan Yüzenada, 21.VI.2015, 9 ♂♂, 8 ♀♀; *C. transcaspicum,* Bingöl, Ilıcalar, 21.V.2015, 14 ♂♂ 13 ♀♀; and *C. orbiculare*, Bingöl, Ilıcalar, 21.V.2015, 13 ♂♂ 15 ♀♀.

Helochares (Mulsant, 1844) species is within the Acidocerinae subfamily, whereas *Coelostoma* (Brullé, 1835) is within the Sphaeridiinae subfamily (Short & Fikácek, 2013).

Helochares obscurus has a body length of 5.0-5.9 mm and a width of 2.6-2.8 mm. Pronotum is 1.2-1.4 mm high and 2.1-2.2 mm wide.

Helochares lividus has a body length of 5.4-5.8 mm and a width of 2.4-2.7 mm. Pronotum is 1.3-1.4 mm high and 2.0-2.1 mm wide.

Coelostoma orbiculare is 4.3-4.9 mm tall and 2.3-2.7 mm wide. Pronotum is 1.0 mm high and 2.2 mm wide.

Coelostoma transcaspicum has a body length of 5.6-5.9 mm and a width of 3.0-3.2 mm. Pronotum is black, short, wide and has a smooth back edge.

Laboratory studies

Extraction of lipids from the samples was carried out by the method of Hara & Radin (1978) using a mixture of 3:2 (v/v) hexane and isopropanol. For this, the tissues were degraded with 10 mL of hexane isopropanol at 3:2 (v/v) in the homogenizer at 11,000 rpm for 30 s. They were then centrifuged for 10 min at 6,000 rpm, and the supernatant from the tissue samples were taken and put into capped test tubes.

Preparation of fatty acid methyl esters

In order to perform gas chromatography analysis on the fatty acids contained in the lipids, they were converted to derivatives such as methyl esters having a non-polar, volatile and stable structure. Although there are different methods for converting fatty acids in lipids to methyl ester derivatives, the acid-catalyzed esterification method described by Christie (1992), which is easy to use and highly efficient, was used.

According to this method; to prepare the methyl esters, the lipid extract in the hexane isopropanol phase was taken up into the 30 ml non-leaking screw cap test tubes. Five ml of 2% methanolic sulfuric acid was added and mixed thoroughly with the vortex. The mixture was left to methylate in an incubator at 50°C for 15 h. After 15 h, the tubes were removed from the incubator, cooled to room temperature, and

mixed thoroughly after adding 5 mL of 5% NaCl. The fatty acid methyl esters formed in the tubes were extracted with 5 ml of hexane and the hexane phase at the top was removed with a pipetted and treated with 5 ml of 2% $KHCO_3$ and left for 4 h to separate the phases. The solvent in the mixture containing methyl esters was then evaporated at 45°C and under nitrogen flow, and the fatty acids at the bottom of the test tubes were dissolved in 1 mL of hexane and transferred into closed autosampler vials for gas chromatography analysis.

After this methylation process, samples were analyzed for fatty acids by Agilent 5975 C model GC/MS gas chromatography device. For this analysis, a Machery-Nagel (Germany) capillary column (30 m x 0.25 mm, 0.25 µm) was used. During the analysis, the column temperature was 140-220ºC, the injection temperature was 250ºC and the detector temperature was 260ºC. Column temperature program was set between 140ºC and 250ºC. Helium (0.5 ml/min) was used as carrier gas. Prior to the analysis of fatty acid methyl esters of the samples, mixtures of standard fatty acid methyl esters (Supelco 37 Component FAME Mix) were injected and the retention times of each fatty acid were determined. After this procedure, necessary programming was done and mixtures of fatty acid methyl esters of the samples were analyzed.

Statistical analyses

Multivariate statistical analyzes were used to reveal the differences between fatty acids in the samples. Plymouth Routines in Multivariate Ecological Research (PRIMER version 7) software was used to establish the relationship between fatty acids and lipid classes. This software is used in the evaluation of ecological data. Multivariate statistical analyzes were performed as analysis of similarities (ANOSIM), evaluation of proportional similarities of non-parametric data (MDS) and percentage of similarities (SIMPER). Global R values obtained from ANOSIM results were used to test the differences between species, subfamilies and genera. Pethybridge et al. (2011) characterized global R values >0.75 as indicating well separated groups, while those <0.25 as barely separated groups. We characterized global R values >0.75 as indicating well separated groups-no difference, <0.25 as barely separated groups-partial difference and <0.1 as indicating unseparated groups-no difference.

Results

Between and in species fatty acid compositions of the samples

Fatty acid analysis was performed on males and females of *Helochares lividus* (n=30), *Helochares obscurus* (n=17), *Coelostoma orbiculare* (n=27) *and Coelostoma transcaspicum* (n=28) collected from the sampling area. A total of 29 fatty acids and 10 common fatty acids were detected in the samples. The most important fatty acids in the samples were 14:0 (myristic acid), 16:0 (palmitic acid), 22:1n-11, 20:1n-9, 20:5n-3 (eicosapentaenoic acid, EPA), polyunsaturated fatty acids: PUFAs, monounsaturated fatty acids: MUFAs, and omega 3 (ω-3) fatty acids (Tables 1 & 2).

Table 1. Fatty acid compositions of *Helochares lividus* and *Helochares obscurus* (%)

Fatty acids (Common Name) (%)	*H. lividus* ♀ (n=16)	*H. lividus* ♂ (n=14)	*H. obscurus* ♀ (n=8)	*H. obscurus* ♂ (n=9)
14:0 (myristic acid)	2.93	2.03	2.43	2.62
i15:0 (pentadecanoic acid isomer)	0.57	0.45	-	-
15:0 (pentadecanoic acid)	0.71	0.38	2.36	1.97
i16:0 (palmitic acid isomer)	2.11	-	2.94	3.42
16:0 (palmitic acid)	22.76	24.79	17.06	17.14
i17:0 (heptadecanoic acid isomer)	0.33	-	-	1.36
17:0 (heptadecanoic acid)	0.57	0.43	1.33	1.51
18:0 (stearic acid)	6.75	12.71	6.58	8.20
20:0 (arachidic acid)	0.35	0.45	-	1.33
Σ SFA (Total Saturated Fatty Acids)	37.08	41.24	32.70	37.55
14:1 (myristoleic acid)	0.38	0.28	-	-
15:1(pentadecaenoic acid)	0.25	-	1.19	-
17:1 (heptadecanoic acid)	0.60	-	-	-
16:1 n-11 (palmitoleic acid)	0.70	2.11	1.14	-
16:1 n-9 (palmitoleic acid)	19.69	11.78	13.12	11.34
18:1n-11 (vaccenic acid)	-	0.91	-	-
18:1n-9 (oleic acid)	13.95	13.6	19.88	19.86
18:1n-7 (vaccenic acid)	1.48	0.67	3.08	3.61
18:1n-6 (vaccenic acid)	-	1.40	-	-
22:1w9 (erucic acid)	1.55	-	-	2.22
Σ MUFA (Total Monounsaturated Fatty Acids)	39.2	30.75	38.41	37.03
16:2n-4 (hecsadecadienoic acid)	1.47	1.22	-	-
18:2 (linoleic acid)	0.91	-	-	-
18:2n-6 (linoleic acid)	9.87	12.3	13.55	13.75
18:2n-4 (linoleic acid)	0.44	1.08	2.78	3.19
18:3n-6 (gamma-linolenic acid)	0.79	0.55	-	-
18:3n-3 (alpha-linolenic acid)	5.14	5.46	5.30	1.46
20:4n-6 (arachidonic acid)	2.18	2.62	2.88	3.52
20:3n-3 (eicosatrienoic acid)	-	-	1.29	-
20:5n-3 (eicosapentaenoic acid)	3.53	3.59	3.06	3.47
Σ PUFA (Total Polyunsaturated Fatty Acids)	24.33	26.82	28.86	25.39
Omega 3 (ω 3)	8.67	9.59	9.66	4.93
Bacterial Fatty acids	5.13	2.91	7.83	8.27
Terrestial Fatty acids (18:3n3+18:2n6)	15.01	17.75	18.85	15.21

Table 2. Fatty acid compositions of *Coelostoma transcaspicum* and *Coelostoma orbiculare* (%)

Fatty acids (%)	C. transcaspicum ♀ (n=13)	C. transcaspicum ♂ (n=14)	C. orbiculare ♀ (n=15)	C. orbiculare ♂ (n=13)
14:0 (myristic acid)	3.16	0.25	2.9	2.8
i15:0 (pentadecanoic acid isomer)	-	0.29	0.43	-
15:0 (pentadecanoic acid)	-	0.38	0.43	0.41
i16:0 (palmitic acid isomer)	2.93	2.83	2.04	1.67
16:0 (palmitic acid)	24.81	22.14	16.70	19.22
i17:0 (heptadecanoic acid isomer)	-	-	2.52	-
17:0 (heptadecanoic acid)	0.37	0.54	0.65	0.64
18:0 (stearic acid)	5.37	-	7,77	7,76
20:0 (arachidic acid)	0,34	0,56	0,45	0,51
Σ SFA (Total Saturated Fatty Acids)	36,98	26,99	33,89	33,01
14:1 (myristoleic acid)	0,36	3,85	0,35	0,34
17:1 (pentadecacanoic acid)	-	0,24	0,28	0,24
16:1 n-11 (heptadecanoic acid)	0,78	0,74	0,73	-
16:1 n-9 (palmitoleic acid)	17,94	18,87	16,98	21,06
16:1 n-7 (vaccenic acid)	-	-	-	0,23
18:1n-11 (vaccenic acid)	0,59	-	20,79	-
18:1n-9 (oleic acid)	24.86	22.96	9.51	16.39
18:1n-7 (vaccenic acid)	2.88	0.73	2.14	0.72
18:1n-6 (vaccenic acid)	-	2.84	0.73	1.61
22:1w9 (erucic acid)	-	2.98	-	-
ΣMUFA (Total Monounsaturated Fatty Acids)	47.41	53.21	51.51	40.59
16:2n-4 (hecsadecadienoic acid)	-	-	-	0.83
18:2 (linoleic acid)	-	0.31	-	0.97
18:2n-6 (linoleic acid))	4.56	4.65	1.28	12.27
18:2n-4 (alpha-linoleic acid)	0.53	0.77	-	0.25
18:3n-6 (gamma-linoleic acid)	0.48	0.76	0.92	1.15
18:3n-3 (alpha-linoleic acid)	3.50	4.63	3.24	2.01
18:3n-4 (octadecatrienoic acid)	0.40	0.48	0.36	-
20:4n-6 (arachidonic acid)	2.44	4.45	4.12	4.31
20:5n-3 (eicosapentaenoic acid)	2.43	3.34	4.37	3.70
Σ PUFA (Total Polyunsaturated Fatty Acids)	14.34	19.39	14.29	25.49
Omega 3 (ω 3)	5.93	7.97	7.62	5.71
Bacterial fatty Acids	3.30	7.13	7.37	4.58
Terrestial Fatty Acids (18:3n3+18:2n:6)	8.06	9.28	4.52	14.28

According to PERMANOVA results, average similarities of fatty acids, within and between species are shown in Table 3. The SIMPER shows the fatty acid similarity in terms of species, genus, gender and subfamily and the contribution on the similarity of each fatty acid in each group.

According to SIMPER, the fatty acid similarity within the Acidocerinae subfamily was 78%. The highest contribution to this similarity was made by palmitic acid (29%), followed by oleic acid (19%) and palmitoleic acid (19%). Fatty acids 18:3n-4, 18:2, and 17:1 made the least contribution to the similarity. The similarity within the Sphaeridinae subfamily was 75%. Palmitic acid (22%), oleic acid (18%) and palmitoleic

acid (17%) made the highest contribution to the fatty acid similarity within the subfamily, whereas the fatty acids with the least contribution were 16:1n-11, 14:1 and 17:1. EPA amount was found to be 4.4%. When we compared the subfamilies of Acidocerinae and Sphaeridinae, the fatty acid similarity ratio was found to be 74%. The fatty acids that contributed most to this similarity were oleic acid (12%), palmitic acid (11%), and linoleic acid (11%), respectively.

Table 3. Fatty acid similarity percentages according to SIMPER and ANOSIM results in the samples (p < 0.01)

| | Average similarity within/between species % | | | | R |
	H. lividus	C. transcaspicum	C. orbiculare	H. obscurus	C. transcaspicum
Helochares lividus	83.10				1.0
Coelostema transcaspicum	76.04	82.98			
Coelostema orbiculare	75.57	71.24	70.99		0.5
Helochares obscurus	77.29	73.42	72.30	90.34	1.0

Figure 1 shows a two-dimensional configuration plot of and MDS analysis of a resemblance matrix of fatty acid data. Multivariate analyses of FA were identified in both of male and female sample means. The samples plotted were factored by species. Stress is called as a degree to which to dimensional configuration plot distorts the sample relationship. If the stress value 0.1, it gives a potentially useful representation. The value must to below 0.05 for an excellent representation (Clarke & Warwick, 2001). Stress value is 0.07 in Figure 1. The value shows a potentially useful representation. The importance of some fatty acids in species gender of the samples. For example, i17:0, 16:1w11, 18:1w7 were more important in female of *C. orbiculare* than the others. Fatty acids 20:2a and 14:1 were more important in male of *C. transcaspicum* than the others.
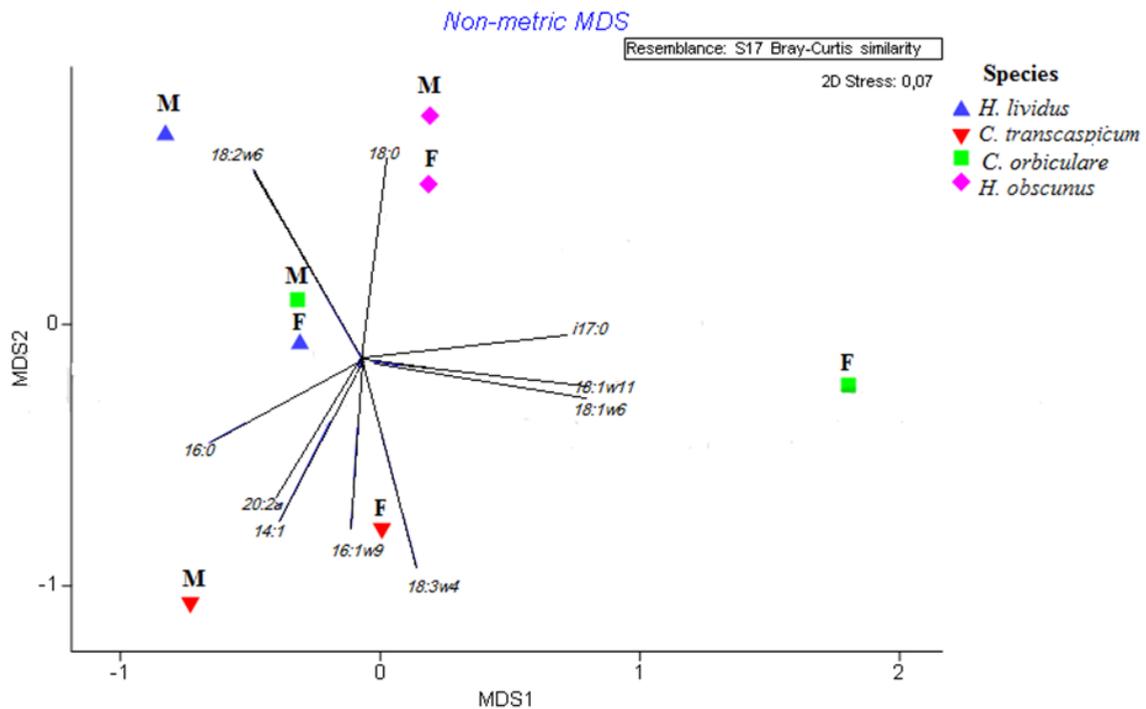

Figure 1. Proportions of fatty acid of the insect samples in a MDS (Multi Dimensional Configuration) plot. Pearson correlations, >0.65, 2D stress 0.07; F: female; M: male.

We found that the average similarity in fatty acid of all female samples in all the species was 74% within gender. The fatty acids that contributed most to this similarity were palmitic acid (24%), palmitoleic acid (20%) and oleic acid (17%), whereas the fatty acids with the least contribution were i17:0, 17:0 and 15:1. Fatty acid similarity rate of all the males in all the species was found to be 75% within the gender. The fatty acids that contributed most to this similarity were palmitic acid (24%), oleic acid (20%) and palmitoleic acid (16%), whereas the fatty acids with the least contribution were 20:2, 20:3n-3 and 22:1n-11. EPA amount in the females was 3.82% whereas that of the males was 4.58%.

Similarity of males and females was found as 76% between genders. Oleic acid (12%), linoleic acid (11%) and 18:1n-11 (11%) had the highest effect on this similarity, whereas 20:1n-9 had the least effect. ANOSIM global R value showed that gender is not statistically significant in fatty acid composition (R=0.08, p < 0.01).

Average similarity of *Helochares* was found to be 78% within the genus. Palmitic acid (29%), oleic acid (19%), and palmitoleic acid (19%) had the highest effect on this similarity, whereas 18:3n-4, 18:2, 17:1 fatty acid had the least effect. Fatty acid similarity of *Coelostoma* was found to be 75% within the genus. Palmitic acid (22%), oleic acid (18%), and palmitoleic acid (17%) had the highest effect on the similarity, whereas 16:1n-11, myristoleic acid and 17:1 had the least effect.

Average similarity in fatty acids of *Helochares* and *Coelostoma* genera was 74% between the species. ANOSIM Global R value revealed that there was a partial difference between these species (R=0.17, p < 0.05).

Palmitic acid, oleic acid and palmitoleic acid contributed the highest on the similarity in *H. lividus, C. transcaspicum* and *C. orbiculare*. However, linoleic acid replaced of palmitoleic acid different from the others with a contribution of 15% in *H. obscurus*.

When we compared to fatty acid composition of *H. lividus* and *C. transcaspicum*, the average similarity between oleic acid and linoleic acid compositions of these species was 73% and the fatty acids with the highest contribution were oleic acid (21%) and stearic acid (15%).

The average similarity was 76% between *H. lividus* and *C. orbiculare* with palmitic acid (12%) and 18:1n-11 (21%) making the highest contribution. Palmitic acid and oleic acid had a 15% and 13% contribution to fatty acid composition.

It was found that 18:1n-9 and 18:1n-11 fatty acids made a 19 and 18% contribution to fatty acid composition similarity between *C. transcaspicum* and *C. orbiculare*, respectively.

When we compared to fatty acid composition of *C. transcaspicum* and *H. obscurus*, linoleic acid had the highest contribution with 17%, followed by palmitic acid (12%) and palmitoleic acid (12%).

When were compared, oleic acid had highest contribution with 19%, followed by linoleic acid (12%) and palmitoleic acid (12%) in *H. obscurus* and *C. orbiculare*. ANOSIM global R values revealed that the difference between species was more significant compared to other factor groups (R=0.63, p < 0.01).

In all species, terrestrial fatty acids predominate. However, it is observed that they are in a higher percentage in Acidocerinae subfamily species than in other species. Fatty acids of terrestrial origin are found in *H. lividus* and *H. obscurus* from the Acidocerinae subfamily at a rate of 17.0 and 16.4%, respectively. However, it is determined that they are also at significant levels in the Sphaeridinae subfamily species. In female *C. transcaspicum* and *C. orbiculare* samples of the Sphaeridinae subfamily, this rate was found to be 4.52 and 8.06%, respectively. When total lipid amounts were compared between species, rates ranging from 12.1 to 48.4% were observed. When *H. lividus* was compared with *H. obscurus*, the highest lipid content was found in *H. lividus*, whereas when male and female samples were compared, the

highest lipid content was again found in males of *H. lividus* with 48%. When *C. transcaspicum* was compared with *C. orbiculare*, the total lipid amount was found to be highest in *C. orbiculare* with 32.3%, and when male and female samples were compared, the highest lipid amount was found in females of *C. orbiculare* with 19.9% (Table 4).

Table 4. Total lipid amount of species according to gender (*Helochares*: 23♂, 24♀; *Coelostoma*: 27♂, 28♀)

	Total lipid (%)	
	♀	♂
Helochares lividus	12.1	48.4
Helochares obscurus	-	-
Coelostema transcaspicum	28.4	32.0
Coelostema orbiculare	19.9	32.3

Discussion

In this study, total lipid and fatty acid composition among (*Helochares* and *Coelostoma)* genera and species of Hydrophilidae (Coleoptera) family collected from Bingöl Province. Our findings indicate that percentage of total lipids differs between genders. This difference is lowest in the males and females of C. *transcaspicum*, and highest in males and females of *H. lividus*. Çakmak et al. (2007) found that total lipid was higher in males than in females. The reason of total lipid levels in female specimens the lower than male may be that females use lipids as a substitute food source for eggs during oogenesis (Ziegler & Antwerpen, 2006). Also, it may be an adaptation mechanism of male insects against low temperature since female insects are reported to be more resistant to low temperatures (Uçkan & Gülel, 2001). In insects, metabolic activities (fatty acid and total fat) vary according to species, gender, nutrition and the habitat (Nurullahoğlu et al., 2004; Çakmak et al., 2007).

A total of 25 fatty acids, consisting of nine saturated, eight monounsaturated and eight polyunsaturated fatty acids, were identified in the females of *H. lividus*. In the males of this species, seven saturated, seven monounsaturated, seven polyunsaturated and a total 21 fatty acids were identified. In the females of *H. obscurus*, a total of 17 fatty acids, consisting of six saturated, five monounsaturated, and six polyunsaturated fatty acids were identified.

High rates of palmitic, palmitoleic, stearic, oleic, arachidonic, linoleic and linolenic acids were detected in the specimens. These findings are consistent with the results obtained for other insect groups (Stanley-Samuelson& Dadd, 1983; Thompson, 1973). Based on our analyses, it was found that the major fatty acids were palmitic acid (16.7-24.8%) and oleic acid (9.51-24.9%). Oleic acid is used in growth and as an energy source in insect groups (Dadd, 1973). Therefore, its levels are generally high in all insects.

Hoback et al. (1999) indicated that adult males and females *of Magicicada septendecim* (Linnaeus, 1758) had similar fatty acid profiles, and both saturated and unsaturated C16 and C18 fatty acids were dominant. In our study, fatty acid composition of males and females did not show statistically any significant difference. Only, in our study, the minor differences in oleic and palmitic acid ratios depending gender, which are the dominant fatty acids, may be due to differences in metabolic activities of these fatty acids in males and females. Oleic acid has been shown to be important in egg production in female insects by demonstrating that oleic acid levels in the ovarium increase in *T. molitor* females during sexual maturity (Khebbeb et al., 1997). The difference in the amount of oleic acid between the genders is thought to be caused by the use of oleic acid for egg formation in females or by the conversion of oleic acid to linoleic acid for this purpose (Nurullahoğlu et al., 2004). We think that the clear difference of oleic acid levels between males and females of *C. orbiculare* in our study could be due to the fact that this species was in

the egg laying period and oleic acid was being used as a substitute food or converted into linoleic acid. In the other species, there was no significant difference in oleic acid levels depending on the genders. The females of *Coelostoma* do not carry the egg sac on the abdomen. However, *Helochares* carries the egg sac on the abdomen (Hansen, 1987). According to our results, this situation did not change the fatty acid results in the insects. In our findings, palmitoleic acid (16:1 n-9) was a predominant unsaturated fatty acid. It was at higher levels in the females of *Helochares* whereas it was higher in the males of *Coelostoma*. The highest percentage (21.1%) was detected in the males of *Coelostoma orbiculare*. It has an important role in pheromone synthesis in many insects (Stanley-Samuelson et al., 1988). In various studies on fatty acids in insects, it has been reported that oleic acid is the most abundant fatty acid in all stages (Nurullahoğlu, 2003; Nurullahoğlu et al., 2004; Seven, 2004; Khani et al., 2007; Üstüner et al., 2010).

Studies with vertebrate and invertebrate revealed that C16:1 n-7 fatty acid is generally in low abundance. The fatty acid has been reported to be at high levels in dipterans (Thompson, 1973), some heteropterans (Spike et al., 1991) and diatoms (Kharlamenko et al., 1995). In our study, C16:1 n-7 was low levels. We think that the Hydrophilidae family can obtain this compound from the algae that constitute their food and from the 16:0 fatty acid. Linoleic acid was significantly higher in *Helochares* than in *Coelostoma*. However, it was significantly lower in *Coelostoma* than in *Helochares*. The fatty acid has been reported to have beneficial effects on human health, such as lipid lowering effects, boosting the immune system, anticarcinogenic, antidiabetogenic and antiatherogenic properties.

Invertebrate and vertebrates synthesize fatty acids up to 18:1n-9, and take 18:2n-6 essential fatty acids with two double bonds and 18:3n-3 essential fatty acids with three double bonds from external nutrients. They synthesize arachidonic acid (ARA, 20:4n-6) and eicosopentanoic acid (EPA, 20:5n-3) from these essential components taken from diets. Especially, prostaglandins members of 20:4n-6 to 20:5n-3 fatty acids eicosanoids have important functions such as reproduction and nodulation in insects (Stanley & Howard, 1998).

Fatty acids 18:2n-6 and 18:3n-3 and their metabolites are precursors of eicosanoids, which are important for insect physiology. Reproduction, cellular immunity and thermoregulation are known to be affected by these eicosanoids (Bozkuş, 2003; Stanley, 2006). Insects can convert 18C polyunsaturated fatty acids to 20C polyunsaturated fatty acids by biochemical processes as all living groups. ARA and EPA are 20C polyunsaturated fatty acids and have a biological importance. They are presented in higher levels in aquatic insects (Stanley-Samuelson et al., 1988). ARA and EPA are presented in aquatic insects higher levels than terrestrial insects. The situation is a result of adaptation mechanism to aquatic environments (Stanley-Samuelson et al., 1988). In this study, the amount of ARA and EPA were high (3.21 and 3.43%, respectively). It was thought that these fatty acids are responsible for immunity against infections and balancing of body temperature. Furthermore, ARA that is synthesized by the elongation and desaturation system from linoleic acid and linoleic acid taken from diets. It was reported that ARA is presented little amounts in some higher terrestrial plants (Shinmen et al., 1991; Shanab et al., 2018) and the major supply of ARA is aquatic organisms (Suloma et al., 2007) especially lower aquatic plants-microalgae (Shanab et al., 2018). Even fishes only heap them by the intake of PUFA-rich microalgae through food-chain (Sayanova & Napier, 2011). Mammals including humans cannot synthesize ARA directly due do the genetic absence of some of its biosynthesis enzymes (Ouyang et al., 2013). The presence of ARA in insects indicates that the insect can synthesize arachidonic acid (Çakmak et al., 2005). However, we think that ARA is of dietary origin because it is found in both the aquatic and terrestrial insects. Çakmak et al. (2005) studied terrestrial insects, so they obtained a different result. Additionally, in our study, it was observed that ARA was present in similar levels in both *Helochares* and *Coelostoma*.

It was observed that the percent distribution of linolenic (18:3-n3) acid, the precursor of eicosanoids and prostaglandins, ranged from 1.46 to 5.46%. This fatty acid is less than 1% in omnivorous insects (Baldus & Mutchmor, 1988). This ratio was found to be higher than 1% in our study, suggesting that although these insect groups are omnivorous, they mainly feed phytophagous, or it may also be related to the fact that these are aquatic insects (Stanley-Samuelson et al., 1990, 1991). In addition, the presence of terrestrial fatty acids in all insect species examined in this study may also indicate that these insects also feed on diets with terrestrial origin. Furthermore, fatty acid results also indicate that bacterial feeding is also at a considerable level (Table 2). Although the majority of Hydrophilidae are aquatic, there are also species living in semiaquatic and terrestrial habitats (Hansen, 1987).

Saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) composition of *Helochares* were found to be similar. It was also found to be a good PUFA source. In the adults of *Coelostoma*, MUFA percentage was higher than SFA. Kalyoncu & Özge (2014), reported similar results showing that MUFA percentage in adult insects was higher than that of SFA. In addition, PUFA percentage in *Coelostoma* was higher in male insects.

In conclusion, within the factor groups (genus, family, subfamily, gender and species) analyzed in the individuals of the Hydrophilidae family in terms of fatty acid composition. ANOSIM results revealed that only gender difference was found to be insignificant for fatty acid composition (R=0.08), whereas differences in subfamilies (R=0.17) and genera (R=0.17) were partially significant, and the difference between species was more significant compared to other factor groups (R=0.63). Furthermore, it was determined that fatty acids cannot be an important biochemical parameter for identifying species in the taxonomy of the Hydophilidae family. This results not valid for all Hydophilidae family. The results include only the studied species of the family. It was determined that the fatty acid composition showed over 70% similarity at the level of these genera and species. The partial difference found in different genders of the same species can be attributed to the regulation of total lipid and fatty acid metabolism according to changing needs.

Acknowledgments

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