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Systematic Evaluation of Fatty Acid Profiles in *Hydrachna processifera*, *Eylais setosa* and *Hydrodroma despiciens* (Acari, Hydrachnidia) Species by GC-MS Method

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Abstract

This study was carried out with *Hydrachna processifera* (Acari, Hydrachnidia), *Eylais setosa* and *Hydrodroma despiciens* common in lakes. Fatty acid ratios were evaluated comparatively in terms of species. For this purpose, these species collected from the Karamık lake (Afyonkarahisar-Turkey) were analyzed by GC-MS gas chromatography in the laboratory. In the results of the analysis, saturated fatty acids such as myristic (C14:0), palmitic (C16:0), heptadecanoic (C17:0), and stearic acid (C18:0) were observed in high amount. There was a significant difference between species in terms of total saturated fatty acid ratios. The monounsaturated fatty acids palmitoleic (C16:1), oleic (C18:1), and erucic (C22:1) are the most common acids. The major polyunsaturated fatty acids were linolenic (C18:3), eicosatrienoic (C20:3), eicosapentaenoic (C20:5) and docosahexaenoic (C22:6) acid, linoleic (C18:2), gamma-linolenic 3), eicosadienoic (C20:2) and arachidonic (C20:4) acids. At the end of the study, there were considerable differences between the species studied in terms of monounsaturated and polyunsaturated fatty acids in these water mite species. This study also found that the fatty acid composition was different for each species and this is important for the molecular taxonomy of water mites.

Keywords: Water mite, Acari, Hydrachnidia, Fatty acid composition.

Abbreviations: GC-MS-Gas Chromatography-Mass Spectrometry

Introduction

Water mites are one of the polyphilic groups in the Acari subclass. They are known as Hydracarina, Hydrachnidia or Hydrachnellae. Over 6.000 species have been defined worldwide, representing 57 families, 81 subfamilies and more than 400 genera. Water mites have a complex life cycle. Their eggs are found on many different water plants in the water. They live in different animal species as ectoparasites in larval stages [1-3].

There is a special significance in the determination of living areas and communities in streams, lakes and ponds. The water mites which spread almost all over inland waters are used as biological indicator organisms in the determination of clean water resources [3-8].

Up to this time, it seems that the studies on the water mites are classical systematic studies. Recently, ecological, genetic, and other molecular studies have also been carried out in this group [9-16].

The determination of fatty acid compositions has been done in water mites (Hydrachnidia) group, for the first time in the present study. In this study, the fatty acid ratios of water mites species were determined and similarities between species have been discussed in terms of these ratios.

Oils are one of the important organic compounds required for all living things, including humans. In addition to being a high energy source, they are very important in terms of combining with proteins to form lipoproteins and to contain fat soluble vitamins [17,18].

Fatty acids are monobasic organic acids with a straight chain and varying chain length, usually containing a double number of carbon atoms. All fatty acids have long hydrocarbon chains, a methyl group at one end of the chain and a carboxyl group at the other end. The presence of long or short chain fatty acids depends on the number of carbon atoms it contains and ranges from 4 to 26. Fatty acids predominantly present in fats usually contain 16-18 carbon atoms. The fatty acids to which all of the carbon atoms are bound by a single bond are called saturated fatty acids (e.g., palmitic acid). Fatty acids containing at least one double bond between carbon atoms are called unsaturated fatty acids (e.g., oleic acid). If there is more than one double bond between carbon atoms, it is called polyunsaturated fatty acid (e.g., linoleic acid) [19].

According to their physical properties, unsaturated fatty acids up to 10°C are present in liquid form at room temperature, while longer chain fatty acids are solid [20].

Studies on fatty acids have been done more in vertebrates, but much less in invertebrate species. In the Acari team, which is an invertebrate subgroup, these studies are seen only in terrestrial forms [21-24].

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Some notable of these are as follows:

The fatty acids contained in some Acari species have been identified in the study entitled: "Variability in cuticular hydrocarbons and phenotypic discrimination of *Ixodes ricinus* populations (Acarina: Ixodidae) from Europe" by Estrada-Pena and coworkers and in the study "Fatty acids as cuticular surface components in oribatid mites (Acari: Oribatida)" by Rasputnig and Krisper [25] and in the study "Cuticular fatty acid profile analysis of three Rhipicephalus tick species (Acari: Ixodidae)" by Shimshoni et al. [26].

No studies have been done about water mites on this subject. This study has been the first on this subject. In this study, it has been seen that the similarities in fatty acid compositions increase with the systematic proximity of water mites.

Materials and Method

Collection of samples

The present study was carried out on water mites *Hydrachna processifera*, *Eylais setosa* and *Hydrodroma despiciens* species (Acari; Hydracnida) collected from Karamik Lake within the boundaries of Afyonkarahisar province. Species determinations were made under the microscope in the laboratory environment.

Determination of fatty acids

Chloroform: Methanol mixture (2:1) was used to obtain oil samples [27]. To obtain crude oil, chloroform:methanol (2:1). 30 ml mixture was added to 0.1 g of the fractionated water mites samples. The samples were then crushed until they became slurry with 24000 rpm ultra-homogenizer. The mixture was filtered with filter paper (1st filtration). The residue on the filter paper was removed and chloroform: methanol mixture (20 ml) was added and homogenized for a second time. The resulting slurry mixture was again filtered. The first and second filtrates were combined.

It was then taken up in a 250 ml separating funnel and 20 ml of reagent solution was added and shaken well and the phases were allowed to stand until separated. The organic phase (chloroform) was taken up in the evaporation flask and the solvent was completely evaporated in the Heidolph-2 brand vacuum rotary evaporator at 45°C. Chloroform remaining in the oil was removed with dry nitrogen and crude oil was obtained.

Esterification process: A 16-20 mg homogenized oil sample was taken in a capped cap and 4 ml of 2% methanolic NaOH solution was added. The tube was sealed with nitrogen gas and then boiled for 10 minutes until saponification occurred on the water bath. At the end of the saponification, 2 ml of 14% BF₃-methanol complex was added to the cooled mixture and boiled for more 5 min.

Then the tube was cooled to 30-40°C and shaken vigorously for 30 seconds by adding of isooctane. 4 mL saturated NaCl solution was added over it. After the mixture was thoroughly shaken, it was taken into the separation funnel and allowed to stand for 5 to 10 minutes to separate the phases. At the end of the extractions, the upper phase (organic phase) was taken and dried with Na₂SO₄. It was then passed through special filters of 0.45 mm in diameter and placed in vials and filled with nitrogen gas to close the caps tightly [28]. This extract was injected into gas chromatography [29].

Injecting the samples into the gas chromatograph: Fatty acid methyl esters were analyzed by gas chromatography (HP Agilent 7890A) using an HP capillary column [(100-m length and 0.25-mm internal diameter and 0.20 µm of film thickness; HP 88)].

Gas chromatographic conditions were follows:

- Injector temperature: 250°C, detector temperature: 250°C, carrier gas: H₂, 30 mL / min.
- Split ratio 50:1, split, flow rate 71.0 ml/min. temperature program, initial 80°C for 50 minutes, temperature 10°C/minute for 40 min at 210°C.

The methylated extract was taken with an automatic injector of gas chromatography and peaks were detected in the chromatograph. The peaks obtained from the samples were identified by comparing the fatty acids with the standard peaks and fatty acids were calculated as percentages.

Fatty acid methyl ester standards: To determine the fatty acids, mix standards containing 37 fatty acids and mix standards containing 4 and 5 fatty acids were used. The fatty acids in the samples were determined by comparing the peaks of the samples with the peaks of the fatty acid standards. Peaks of fatty acids in the standard are shown in Figure 1.

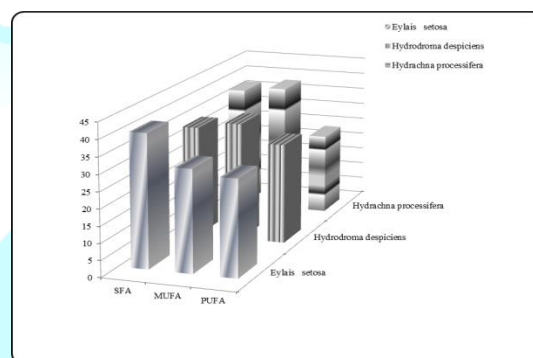


Figure 1: Average SFA, MUFA and PUFA ratios (%) for species living in Karamik Lake.

Statistical evaluation: Statistical analyses were performed with the SPSS 18.0 computer program. First, the normality test of the data was performed. Single and two-way analysis of variance was used because the data showed normal distribution and there were more than two groups. The Turkey test was used for multiple comparison tests of homogeneous groups and the Tamhane test was used for the non-homogenous groups by controlling the homogeneity of the variances. Also, the arithmetic average \pm standard deviation values were given for each group.

Results and Discussion

This study was carried out with common species of water mites (Acari, Hydracnida) *Eylais setosa*, *Hydrodroma despiciens*, and *Hydrachna processifera* in lakes. These three species are included in a separate water mite family group. In the present study, fatty acid compositions were determined for the first time in water mites species.

No studies have been found on the identification of fatty acid compositions in the water mites until now. However, few studies have been made on this issue with some terrestrial and parasitic species of Acari. Among these, some notable studies are as follows:

In their study, Aboshi et al. have reported that *Tyrophagus similis* and *Tyrophagus putrescentiae* (Astigmata: Acaridae) species have the ability to biosynthesize linoleic acid [(9Z, 12Z) -9, 12-octadecadienoic acid].

Murungi and colleagues reported that fatty acids in essential oils (camphor, limonene, decanoic acid, hexadecanoic acid, dodecanoic acid) from the leaves and fruits of *Solanum sarrachoides* plant had



negative effects on the laying of *Tetranychus evansi* (tomato spider mite) [30].

In another study, Maazouzi et al. investigated the effects of diarrhea and feeding on the hepatopancreatic fatty acid composition of the *Eriocheir sinensis* species at different periods and they reported that total saturated and monounsaturated fatty acids differed by nutritional status [31].

In a similar study, Wen et al., showed that fatty acid analysis of phospholipids, neutral lipids and total lipids of the *Unio elongatulus* species showed the highest C16:0 (18.23% -24.86% 9 (10.23% -45.10%) and C18:2n-6 (3.50% -16.94%) fatty acids [32]. In the study of Wen et al., the ratio of total polyunsaturated n-3 and n-6 PUFA (43.86%) to phospholipid; the proportion of total monounsaturated fatty acids (61.39%) was found to be high in neutral lipid.

Another important study on this subject was carried out by Hayashi and Takagi [33]. In this study, researchers found that saturated fatty acids such as myristic, palmitic and stearic acid taken via food and unsaturated fatty acids such as oleic, linoleic and linolenic acid were stored directly in fish oils and that the seasonal variation of these fatty acids was due to the phytoplankton, zooplankton [33].

In the present study titled "Fatty acid and lipid composition of Water Mites (Acari, Hydracnida) species by GC-MS", fatty acid ratios in the waters (*Eylais setosa*, *Hydrodroma despicens* and *Hydrachna processifera*) are evaluated as saturated, monounsaturated and polyunsaturated in **Tables 1-3**.

Saturated and unsaturated fatty acid results

In this study, oil acid analysis was carried out by gas chromatography (GC-MS) in water mites (Acari, Hydracnida) species *Hydrachna processifera*, *Eylais setosa* and *Hydrodroma despicens* collected from Karamik Lake. The results obtained were evaluated as saturated monounsaturated and polyunsaturated fatty acids. The data were statistically evaluated and included with standard deviations in **Table 1**.

Results of saturated fatty acids: From **Table 1**, it is seen that palmitic (C16:0) and stearic acid (C18:0) of saturated fatty acids are found in high proportion in three of the water mites samples studied. According to these numerical values, there is a remarkable difference between species in terms of palmitic and stearic acid.

Fatty acid	<i>Eylais setosa</i>	<i>Hydrodroma despicens</i>	<i>Hydrachna processifera</i>
C6:0	Nd	Nd	Nd
C8:0	0.161 ± 0.012	0.557 ± 0.024	0.175 ± 0.067
C10:0	0.223 ± 0.034	0.154 ± 0.034	0.243 ± 0.047
C11:0	Nd		0.144 ± 0.031
C12:0	0.739 ± 0.071	0.707 ± 0.028	0.817 ± 0.154
C13:0	0.323 ± 0.165	0.083 ± 0.016	0.227 ± 0.072
C14:0	2.356 ± 0.312	2.343 ± 0.099	2.881 ± 0.326
C15:0	0.329 ± 0.020	0.350 ± 0.013	0.586 ± 0.266
C16:0	12.444 ± 0.100	15.372 ± 0.242	16.624 ± 0.326
C17:0	1.733 ± 0.060	0.841 ± 0.537	1.150 ± 0.136
C18:0	19.92 ± 1.202	11.941 ± 0.202	13.112 ± 0.600
C20:0	0.448 ± 0.203	0.333 ± 0.011	0.331 ± 0.029
C21:0	Nd	Nd	Nd
C22:0	0.714 ± 0.281	0.206 ± 0.105	0.456 ± 0.097
C23:0	0.333 ± 0.052	0.118 ± 0.025	0.335 ± 0.088
C24:0	0.347 ± 0.080	0.152 ± 0.061	0.297 ± 0.122

Table 1: Average saturated fatty acid compositions (%) of water mites species in Lake Karamik.

The ratio of total saturated fatty acid was found 40.072% for *Eylais setosa*, while *Hydrodroma despicens* 33.299% and *Hydrachna processifera* 37.235% were found in other species. There was a

significant difference in SFA ratios between species. The change in total SFA rates is shown in **Table 1** and **Figure 1**.

The ratio of stearic acid was found to be 11.941% in the lowest *Hydrodroma despicens* and 19.924% in the highest *Eylais setosa*. There was no significant difference found between *Hydrodroma despicens* and *Hydrachna processifera* when compared to the other two species in *Eylais setosa* in terms of stearic acid ratio.

The lowest percentage of palmitic acid was found in *Eylais setosa* (12.444%) and the highest in *Hydrachna processifera* (16.624%). There was a significant difference in palmitic acid ratio compared to the other two species of *Eylais setosa*, but no difference was observed between *Hydrodroma despicens* and *Hydrachna processifera*.

Monounsaturated fatty acid results: The changes in the monounsaturated fatty acid compounds in the samples are shown in **Table 2** and **Figure 1**.

Fatty acid	<i>Eylais setosa</i>	<i>Hydrodroma despicens</i>	<i>Hydrachna processifera</i>
	Ort. S. Sapma	Ort. S. Sapma	Ort. S. Sapma
C14:1	0.437 ± 0.204	0.157 ± 0.008	0.513 ± 0.406
C15:1	0.193 ± 0.028	0.064 ± 0.019	0.159 ± 0.050
C16:1	5.853 ± 0.209	5.325 ± 0.079	5.607 ± 0.156
C17:1	0.516 ± 0.062	0.470 ± 0.020	0.725 ± 0.116
C18:1n9t	0.234 ± 0.033	0.145 ± 0.030	0.239 ± 0.067
C18:1n9c	20.651 ± 0.702	27.078 ± 0.213	28.340 ± 0.271
C20:1	0.226 ± 0.081	0.448 ± 0.036	0.244 ± 0.082
C22:1n9	2.683 ± 0.054	1.541 ± 0.077	2.510 ± 0.202
C24:1	Nd	0.132 ± 0.027	0.265 ± 0.090

Nd: Below the detection limit

Table 2: Average monounsaturated fatty acid compositions (%) of water mites species in Lake Karamik.

Among the monounsaturated fatty acids, the most abundant fatty acids are palmitoleic acid (C16:1), oleic acid (C18:1) and erucic acid (C22:1). The total monounsaturated fatty acid content was found to be 38.638% in *Hydrachna processifera*, 30.792% in *Eylais setosa* and 35.400% in *Hydrodroma despicens*. There was a significant difference in the MUFA ratios between the species.

Palmitoleic acid ratio was 5.325% in *Hydrodroma despicens* and 5.853% in *Eylais setosa*. In terms of palmitoleic acid ratios, there were no significant differences detected between the species.

The lowest oleic acid content was found in *Eylais setosa* (20.651%) and the highest in *Hydrachna processifera* (28.340%). In terms of oleic acid ratio, there was a significant difference in comparison with the other two species of *Eylais setosa*, but no difference was observed between *Hydrodroma despicens* and *Hydrachna processifera*.

Erucic acid ratio was found to be 1.541% in *Hydrodroma despicens* and 2.683% in *Eylais setosa*. In terms of erucic acid ratios, *Hydrodroma despicens* showed a significant difference when compared to the other two species, but no difference was observed between *Eylais setosa* and *Hydrachna processifera*.

Polyunsaturated fatty acid results: The main polyunsaturated ω3 fatty acids are linolenic acid (C18:3ω3), Eicosatrienoic acid (C20:3ω3), Eicosapentaenoic acid (C20:5ω3) and Dokosaheptaenoic acid (C22:6ω3). ω6 fatty acids are Linoleic acid (C18:2ω6), γ-Linolenic acid (C18:3ω6), Eicosadienoic acid (C20:2ω6) and Arachidonic acid (C20:4ω6).

The total polyunsaturated fatty acid ratio was found to be 30.076% in *Hydrodroma despicens*, 29.122% in *Eylais setosa* and 24.238% in *Hydrachna processifera*. There was a significant difference in PUFA ratios between species. The variation of the total PUFA ratios is shown in **Table 3**.



Fatty acid	<i>Eylais setosa</i>	<i>Hydrodroma despicens</i>	<i>Hydrachna processifera</i>
C18:2n6t	0.610 ± 0.095	0.231 ± 0.115	0.402 ± 0.157
C18:2n6c	13.063 ± 0.118	17.137 ± 0.260	10.452 ± 0.376
C18:3n6g	0.916 ± 0.088	1.209 ± 0.078	1.133 ± 0.114
C18:3n6	5.057 ± 0.171	5.388 ± 2.610	4.734 ± 1.892
C18:3n3	0.449 ± 0.098	0.194 ± 0.039	0.301 ± 0.073
C20:2	0.649 ± 0.173	0.204 ± 0.031	0.334 ± 0.120
C20:3n6	0.457 ± 0.333	0.193 ± 0.075	0.380 ± 0.108
C20:3n3	0.266 ± 0.060	0.524 ± 0.044	0.281 ± 0.052
C20:4n6	0.252 ± 0.104	0.135 ± 0.044	0.336 ± 0.087
C22:2	6.333 ± 0.044	4.188 ± 0.097	4.946 ± 0.559
C20:5n3	0.248 ± 0.081	0.098 ± 0.052	Nd
C22:5n3	0.218 ± 0.047	0.153 ± 0.036	0.341 ± 0.076
C22:6n3	0.604 ± 0.162	0.422 ± 0.023	0.598 ± 0.321

Nd: Below the detection limit

Table 3: Average polyunsaturated fatty acid compositions (%) of water mites species in Lake Karamik.

In $\omega 3$ fatty acids, eicosatrienoic acid was found to be the highest in *Hydrodroma despicens* at 0.524% and the lowest in *Eylais setosa* at 0.266% and 0.281% in *Hydrachna processifera*. *Hydrodroma despicens* in terms of eicosatrienoic acid ratio was significantly different when compared to the other two species, but no difference was observed between *Eylais setosa* and *Hydrachna processifera*.

Eicosapentaenoic acid in $\omega 3$ fatty acids was found to be the highest in *Eylais setosa* at 0.248%, 0.098% in *Hydrodroma despicens* and below the detection limit in *Hydrachna processifera*. When compared to *Eylais setosa* and *Hydrodroma despicens* in terms of eicosapentaenoic acid ratios, significant differences were observed.

The highest concentration of docosahexaenoic acid was found in *Eylais setosa* (0.604%) and the lowest level was found in *Hydrodroma despicens* (0.422%) and in *Hydrachna processifera* (0.598%).

Species Names	% Fatty acid				
	Palmitik asit	Stearik asit	Palmitoleik asit	Oleik asit	Erusik asit
<i>Eylais setosa</i>	12.444 ± 0.100	19.92 ± 1.202	5.853 ± 0.209	20.651 ± 0.702	2.683 ± 0.054
<i>Hydrodroma despicens</i>	15.372 ± 0.242	11.941 ± 0.202	5.325 ± 0.079	27.078 ± 0.213	1.541 ± 0.077
<i>Hydrachna processifera</i>	16.624 ± 0.326	13.112 ± 0.600	5.607 ± 0.156	28.340 ± 0.271	2.510 ± 0.202
<i>Rhipicephalus sanguineus</i> 1	42.2 ± 5.6	41.1 ± 5.6	-	2.6 ± 0.4	-
<i>Rhipicephalus bursa</i> 1	30.1 ± 2.5	36.2 ± 4.2	-	8.0 ± 2.7	-
<i>Rhipicephalus annulatus</i> 1	33.2 ± 2.1	51.1 ± 6.5	-	6.5 ± 2.1	-
<i>Varroa destructor</i> 2	22.62 ± 0.87	11.22 ± 0.87	1.61 ± 0.13	41.07 ± 2.26	ND
<i>Tenebrio molitor</i> Larvae3	21.33 ± 0.13	7.92 ± 0.07	1.97 ± 0.01	35.83 ± 0.33	0
<i>Acheta domesticus</i> 3	22.65 ± 0.37	8.54 ± 0.00	0.34 ± 0.00	2.18 ± 0.02	0.52 ± 0.01
<i>Chorthippus parallelus</i> 3	11.97 ± 0.06	13.34 ± 0.05	0.28 ± 0.20	14.85 ± 0.09	0
<i>Conocephalus discolor</i> 3	10.03 ± 0.08	6.86 ± 0.18	1.92 ± 0.12	19.09 ± 0.12	0
<i>Cyprinus carpio</i> 4	16.79 ± 0.89	5.11 ± 0.35	10.78 ± 0.75	17.93 ± 0.90	-
<i>Esox lucius</i> 5	18.98 ± 0.4	5.09 ± 0.30	5.57 ± 0.50	10.77 ± 0.60	-

Table 4: Comparison of fatty acid ratios in different species (%).

It appears that the proportions of species close to each other. It is also seen that the proportions of fish and water mites species collected from the same locality are relatively similar. This can be explained by the same locality and seasonal cycle. On the other hand, when we look at the studies on other terrestrial parasites (*Rhipicephalus sanguineus*, *Rhipicephalus bursa*, *Rhipicephalus annulatus*), it is seen that each fatty acid composition compared to the water mites is quite variable for all three species given in the table. The main reason for this is that each of these parasite species is found as a host on a different animal.

When the fatty acid compositions of each species were examined in four different insect species belonging to insect class (*Tenebrio molitor* Larvae, *Acheta domesticus*, *Chorthippus parallelus*, *Conocephalus discolor*) where there is a great similarity in species belonging to the same team species there is a big difference found between the ratios of both teams. This is due to the fact that these three species belong to different teams of the insect class and therefore differ in their systematic distance and nutrition patterns. According to the table, another noteworthy situation is that the fatty acid composition ratios in two different species of fish caught in the same locality (*Cyprinus*

Hydrodroma despicens in terms of docosahexaenoic acid ratio was significantly different when compared to the other two species, but no difference was observed between *Eylais setosa* and *Hydrachna processifera*.

Linolenic acid content was highest in *Eylais setosa* (0.449%) and lowest in *Hydrodroma despicens* (0.194%) and in *Hydrachna processifera* (0.301%). Significant differences were observed between species in terms of linolenic acid ratios. The highest ratio of arachidonic acid in $\omega 6$ fatty acids was 5.388% in *Hydrodroma despicens* while the lowest was 4.734% in *Hydrachna processifera* and 5.057% in *Eylais setosa*. There were no significant differences found between the species in terms of arachidonic acid ratios.

Among the $\omega 6$ fatty acids, C18:2 $\omega 6$ was found to be the highest in *Hydrodroma despicens* at 17.137% and the lowest in *Hydrachna processifera* at 10.452% and 13.063% in *Eylais setosa*. There was a significant difference between species in terms of C18:2 $\omega 6$ ratios.

Among $\omega 6$ fatty acids, the highest ratio of C22:2 $\omega 6$ was 6.333% in *Eylais setosa* and the lowest was 4.188% in *Hydrodroma despicens* and 4.946% in *Hydrachna processifera*. There was no significant difference between *Hydrodroma despicens* and *Hydrachna processifera* when compared to the other two species of *Eylais setosa* in terms of C22:2 $\omega 6$ ratio.

Fatty acid compositions of different species are given comparatively in Table 1. In terms of the fatty acid (SFA, MUFA and PUFA) ratios found, the values of water mites species (*Hydrodroma despicens*, *Hydrachna processifera*, *Rhipicephalus sanguineus*) were found to be close to each other. This was due to systematic closeness. **Table 4** gives the fatty acid ratios in different species.

carpio, *Esox lucius*) are surprisingly similar. In this case, these observed results for these two species can also be attributed to habitat similarity, seasonal characteristics and nutritional factors.

As a result, it can be said that each of the fatty acid compositions is unique, and that systemic affinity, habitat and seasonal cycles are also effective on these fatty acid compositions. All the results of this study showed that this method can help to solve future taxonomic problems in water mites.

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