

## ON THE RELATIONSHIP BETWEEN THE OCCURRENCE OF OSTRACOD SPECIES AND ELEVATION IN SAKARYA PROVINCE, TURKEY

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In order to determine the distribution of ostracods species between sea level and 1133 m a.s.l. in the Sakarya Province of Turkey, 83 different aquatic sites were randomly sampled during 9–12 May, 2014. This sampling yielded a total of 9598 individuals belonging to 33 taxa (31 living). The species *Vestalenula cuneata* and *Kovalevskiella phreaticola* were new reports for Turkey. The number of species with males (19) and without males (12) tends to decrease with increasing elevation while there is no significant difference in the number of species per site. This corresponds to a decrease in the numbers of species with (14) and without (17) natatory setae on second antennae (A2). Accordingly, presence of species with/without natatory setae on A2, with different reproductive modes, and numbers of species changed along with elevation seem to be all mutually independent in the population sampled. The first two axes of Canonical Correspondence Analysis explained 74.4% of relationships between species and environmental variables. Furthermore, two well-known cosmopolitan species (*Heterocypris incongruens* and *Ilyocypris bradyi*) were observed in almost all of the elevational ranges. Overall, these results support the prediction that elevation seems to have no direct influence on the presence of species with or without swimming setae in sexual or parthenogenetic populations. Results suggest that unlike non-cosmopolitan ostracods, species with cosmopolitan characteristics may not be limited by elevation.

Keywords: ostracod, Kolmogorov-Smirnov, elevation, distribution, tolerance.

### INTRODUCTION

The main categories of biodiversity drivers were classified as climate (variation in abiotic variables), space (species–area relationship), evolutionary history (speciation and extinction rates, clade age and phylogenetic niche conservatism) and biotic processes (ecotone effects, competition, mutualisms, habitat heterogeneity and habitat complexity) (McCain & Grytnes 2010). Besides, the extinction risk of species is determined by the ecological and climatic limitations and also by the effect of elevation on their physiology (Şekercioğlu *et al.* 2008). Thereby, the survival and occurrences of species depends on their ecological tolerance ranges (e.g., temperature) to different levels of environmental variables and their fluctuations caused by climate change (Thomas *et al.* 2004). Microclimatic changes in an area may be positively correlated with

elevation (MERRIAM 1890, STEVENS 1992). Reduction in air density and pressure with increasing elevation result in an air temperature decrease (BROWN & GIBSON 1983). This decrease in air temperature can eventually lower the water temperature. Indeed, gradual decrease of water temperature in the Tibetan region from 2668 to 5133 m a.s.l. was emphasized by VAN der MEEREN *et al.* (2010). Similarly, PREUD'HOMME & STEFAN (1992) showed the response of water temperatures of lotic environments to air temperatures according to the depth of rivers. This phenomena is directly or indirectly affecting aquatic organisms by means of changing dissolved oxygen concentration and habitat loss (LIVINGSTONE & LOTTER 1998). This is because temperature is a deterministic factor for the geographic ranges of many aquatic and wetland species (POFF *et al.* 2002). Accordingly, fluctuation in physico-chemical variables of aquatic bodies related to water temperatures changes with the ascending or descending of elevation (REEVES *et al.* 2007, ROGORA *et al.* 2008) may affect the occurrence and distribution of species (e.g., ostracods). In addition, ROBINSON *et al.* (2008) indicated that elevation as a predictor variables of pH and nitrate (low pH and high nitrate at higher elevations) in Great Smoky Mountains National Park (U.S.A). In that case, species with wider tolerance levels to ecological variables and higher mutational ratios showed much more advantages for adapting to changing environments when we compared with others.

Ostracods are small (0.3–5 mm in length) bivalved microscopic organisms that are found in a variety of marine and non-marine aquatic bodies (MEISCH 2000). They show a wide distribution in the world (MARTENS *et al.* 2008, 2013) by means of using active and/or passive distribution modes (MEISCH 2000, AKDEMİR *et al.* 2016). Small location change in the same habitat can be done actively with the aids of swimming setae of the individuals (KÜLKÖYLÜOĞLU 2013). In contrast, dispersion can be achieved passively by different agents such as human, birds, fishes, wind (resting eggs), plants, amphibians and insects to longer distance (MCKENZIE & MORONI 1986, HORNE & MARTENS 1998, FIGUEROLA *et al.* 2003, GREEN *et al.* 2008, VANSCHOENWINKEL *et al.* 2008, RODRIGUEZ-LAZARO & RUIZ-MUÑOZ 2012, GREEN 2016, VALLS *et al.* 2016). In addition to biotic factors, their distribution can also be depending and/or limited on abiotic (e.g., pH, elevation) factors. The effect of elevation is a long-term debate in the literature. Some studies stated that elevation was a limiting factor for ostracods (e.g., MEZQUITA *et al.* 1999, PIERI *et al.* 2009), zooplankton (SHEHU *et al.* 2009) and avian species (ŞEKERCİOĞLU *et al.* 2008) but others (e.g., LAPRIDA *et al.* 2006, KÜLKÖYLÜOĞLU *et al.* 2012a, GUO *et al.* 2013, YAVUZATMACA *et al.* 2015) did not support this idea. The elevational limitation of vectors (e.g., birds) that contributes to the dispersion of ostracods to long distance may allow to any one say that elevation is a secondary factor (MARTINEZ-GARCIA *et al.* 2015, YAVUZATMACA *et al.* 2015) limits the passive distribution of ostracods. The controversy clearly outlines the fact that there is a need for future studies

dealing with relationships between the effect of elevation on the distribution and occurrence of ostracods. Hence, the main objective of study is to determine the distribution of ostracods across an elevation range of 0-1133 m a.s.l. in the Sakarya province of Turkey. Based on this main objective, we wanted to test the following hypotheses; i) Number of ostracod species are distributed uniformly from sea level to elevation of 1133 m a.s.l., ii) Numbers of ostracod species per site are distributed uniformly from sea level to elevation of 1133 m a.s.l., (in both (i and ii) hypotheses, we questioned how likely distribution of ostracod species (or species per site) from random observed data as specified in the null hypotheses) and iii) Occurrence of swimming setae on A2, presence of bisexual population with males and changes in elevation ranges are all mutually independent in the population sampled.

## MATERIAL AND METHODS

### *Study area and sampling*

Sakarya Province (40°17'-41°13'N, 29°57'-30°53'E, 4817 km<sup>2</sup> of surface area) is located in the north east side of Marmara region of Turkey (SAKARYA İKTM 2018). North side of the province is bordered by Black Sea. Total of 83 randomly selected sampling sites (one sample from each site) including seven different habitat types (spring, pond, pool, lake, slough, trough and creek) were visited once during 9-12 May of 2014 in Sakarya province (Fig. 1). We collected ostracod samples from about 1 m<sup>2</sup> area with a maximum of 1 m depth from each site. To obtain the accurate value of environmental variables, water quality measurements were recorded before sampling. A YSI-Professional Plus was used to record dissolved oxygen concentration (DO, mg/L), percent oxygen saturation (%DO.), water temperature (Tw, °C), electrical conductivity (EC, µS/cm), pH, atmospheric pressure (mmHg) and oxidation-reduction potential (ORP, mV; measured directly from water). We recorded air temperature (°C) with a Testo 410-2 model anemometer while a GARMIN e-trex Vista H GPS was used to obtain geographical data (elevation, coordinates).

Sediments including ostracods were collected with a standard sized hand net (200 µm in mesh size) from the littoral region of lentic and slow flowing parts of lotic environments. All collected samples were fixed with 70% of ethanol in 250 ml plastic bottles *in situ* to prevent the deterioration of ostracod specimens. In laboratory, ostracods samples washed and filtered through four standardized sieves (0.5, 1.0, 1.5 and 2.0 mm mesh size) under pressured water. Subsequently, the washed samples were put again into 250 ml plastic bottles and fixed with 70% ethanol for long term storage. Sorting of ostracods from the sediments were done under a stereomicroscope (Olympus ACH 1X) by using a pipette and dissecting needles. Taxonomic identification of species based on carapace morphology and dissecting soft body parts in lactophenol solution under a light microscope (Olympus BX-51) was done by following the taxonomic keys provided in MEISCH (2000) and KARANOVIC (2012). Scanning Electron Microscope (JEOL JSM-6390LV SEM) photographs of some species were taken at Abant İzzet Baysal University. Ostracod samples can be available upon request and that are stored at the Limnology Laboratory of Abant İzzet Baysal University, Bolu, Turkey.

### Statistical analysis

Kolmogorov-Smirnov (K-S) Goodness of Fit ( $D$  (test statistics) is the largest value of  $D_i$  or  $D'_i$ ) and  $\delta$ -corrected K-S Goodness of Fit (if  $n \leq 25$ ,  $D$  is the largest value of  $D_{0,i}$  or  $D_{1,i}$ ) tests (LILLIEFORS 1967, DALLAL & WILKINSON 1986, ZAR 1999) were used to test the vertical distributions of ostracod species and species per site from sea level to 1133 m a.s.l. (for calculation of both tests see Supplementary Tables S1 and S2). If  $D > D_{\alpha, n}$  reject null hypothesis ( $H_0$ ). A Chi-square analysis (ZAR 1999) was used to test for mutual independence in three dimensional contingency table among elevation, occurrence of natatory setae on second antennae (A2) and presence of male (see Table S3). The range of elevation herein is 1133 and we broke this range into a number of equal elevational interval with a width of 100 m for these previous analyses. Therefore, the range is divided by width of intervals ( $1133/100 = 11.33$ ) as a result 11 elevational intervals were created (see Tables 2 and S4, Note: one site above 1100 m included among the 1001–1100 m ranges). The ecological tol-

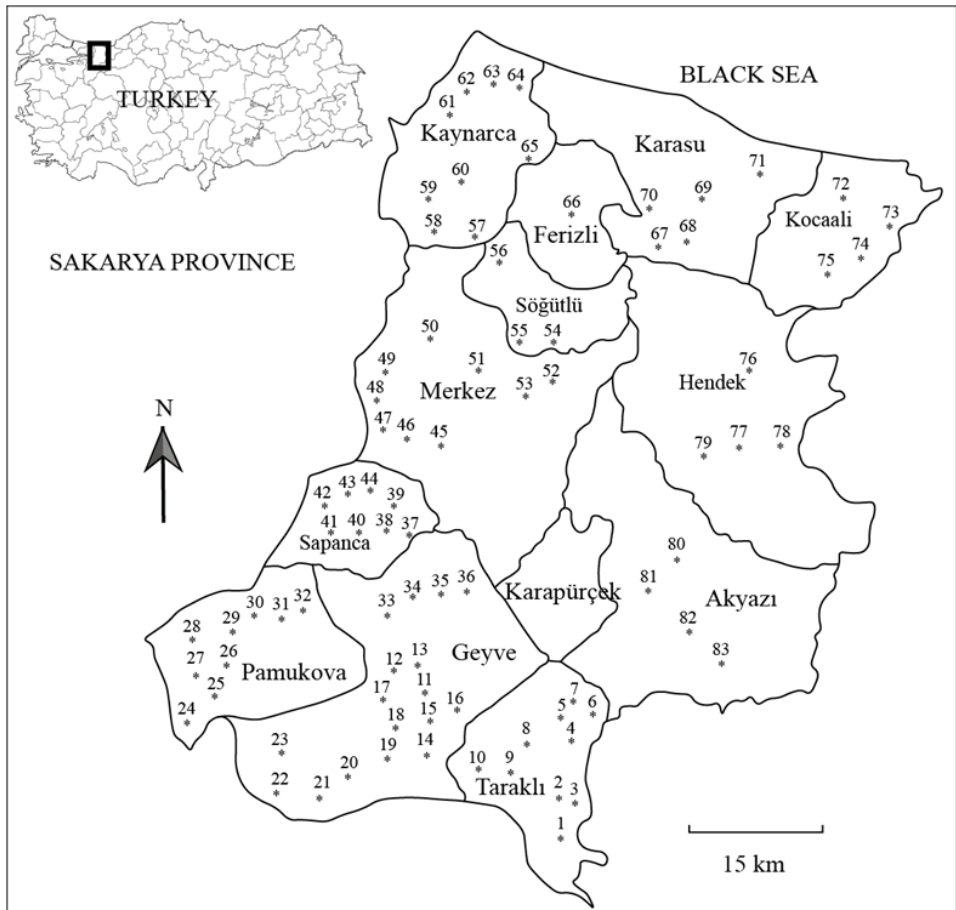


Fig. 1. Location of sampling sites in 13 counties of Sakarya province

**Table 1.** Taxa recorded in Sakarya province and species with/without males and A2 natory setae. \* represents new records for Sakarya province. Species names in bolditalics show cosmopolitan distribution.

Taxa	Code	with male	without male	with natory setae	without natory setae
1 * <i>Aurila</i> sp.	As				
2 <b><i>Darwinula stevensoni</i></b>	Ds		+		+
3 * <i>Vestalenula cuneata</i>	Vc		+		+
4 * <i>Paracandona</i> sp.	Ps				
5 <b><i>Candona neglecta</i></b>	Cn	+			+
6 <i>Candona angulata</i>	Ca	+			+
7 * <i>Pseudocandona hartwigi</i>	Ph	+			+
8 <i>Candonopsis kingsleii</i>	Ck	+			+
9 <b><i>Physocypris kraepelini</i></b>	Pk	+		+	
10 <i>Cypria ophtalmica</i>	Co	+		+	
11 <i>Cypridopsis vidua</i>	Cv		+	+	
12 * <i>Cyclocypris ovum</i>	Cov	+		+	
13 <b><i>Prionocypris zenkeri</i></b>	Pz		+		+
14 <i>Eucypris virens</i>	Ev	+		+	
15 <b><i>Heterocypris incongruens</i></b>	Hi	+		+	
16 <b><i>Heterocypris salina</i></b>	Hs		+	+	
17 * <b><i>Heterocypris reptans</i></b>	Hr	+			+
18 <b><i>Ilyocypris bradyi</i></b>	Ib		+		+
19 * <i>Ilyocypris inermis</i>	Ii		+		+
20 * <i>Ilyocypris monstifca</i>	Im	+		+	
21 <b><i>Ilyocypris gibba</i></b>	Ig	+		+	
22 * <i>Ilyocypris getica</i>	Ige	+			+
23 <b><i>Psychrodromus olivaceus</i></b>	Po	+			+
24 <b><i>Herpetocypris chevreuxi</i></b>	Hc		+	+	
25 * <i>Herpetocypris intermedia</i>	Hin		+		+
26 <b><i>Potamocypris villosa</i></b>	Pv	+		+	
27 * <i>Potamocypris arcuata</i>	Pa	+		+	
28 * <i>Potamocypris fulva</i>	Pf		+		+
29 * <i>Potamocypris fallax</i>	Pfa		+		+
30 * <i>Notodromas monacha</i>	Nm	+		+	
31 * <i>Notodromas persica</i>	Np	+		+	
32 * <i>Kovalevskiella phreaticola</i>	Kp		+		+
33 * <i>Metacypris cordata</i>	Mc	+			+

erance ( $t_k$ ) and optimum ( $\mu_k$ ) values of species for different environmental variables were calculated by C2 software (JUGGINS 2003) when Canonical Correspondence Analysis (CCA, after Detrended Correspondence Analysis) was performed by software package CANOCO for Windows 4.5 to explain the relationships between species and environmental variables (TER BRAAK 1987). In both (tolerance and optimum, and CCA) analyses, species with three or more occurrences were used. The levels of correlations among species, and environmental variables were calculated by a non-parametric Spearman Rank Correlation analysis (IBM-SPSS Statistics Version 21). In all analyses, adult individuals with complete soft body parts and carapaces were used while empty carapaces, valves and juveniles were omitted.

## RESULTS

Total of 9598 individuals belonging to 33 taxa (31 recent and 2 sub-recent) were reported from 63 of 83 sampling sites (Table S4). In total, 19 of 31 species reproduce both sexually (males and females present) and parthenogenetically (only females present) but the rest show only parthenogenetic (without males) reproduction. More than half (11) of species with males have well developed A2 natatory setae (setae that at least reach the tips of the terminal A2 claws but reduced or undeveloped setae do not reach beyond the base of the terminal claws) while 1/4 of species without males (3) have well developed natatory setae (see Table 1).

The number of species and species with/without males and natatory setae tend to fall when the elevational ranges increased from sea level to 1133 m a.s.l. but only number of species per site showed a tendency to increase (see Table 2).

The hypothesis, "Number of ostracod species are distributed uniformly from sea level to elevation of 1133 m a.s.l." was rejected ( $D = 0.28$  (largest value of  $D_i$  or  $D'_i$ )  $> D_{0.05(2),67} = 0.16$ ). This means species number ( $f_i$ ) did not show a regular distribution when elevation increased (Table S1). Correspondingly, species number showed variability, such as, 21 species at 0–100 m, 7 species at 401–500 m, 3 species at 1001–1100 m.

In contrast to first hypothesis, "Number of ostracod species per site are distributed uniformly from sea level to elevation of 1133 m a.s.l." was accepted ( $D = 0.18$  (largest  $D_{0,i}$  or  $D_{1,i}$ )  $< D_{0.8(\text{Table})} = 0.37$ ). This means numbers of species per site did not show significant difference among increased elevational ranges (Table S2) even though sampling sites and encountered species number fluctuations (e.g., 9 sites and 8 species at 101–200 m, 8 sites and 7 species at 401–500 m, 5 sites and 7 species at 801–900 m and for more see Table 2).

The last hypothesis "Occurrence of natatory setae on A2, presence of bisexual population with males and changes in elevational ranges are all mutually independent in the population sampled" meaning that there are no interactions either of three-way or two-way among of these variables was accepted (Chi-square  $_{\text{table}(0.05, 31)} = 44.99 > \text{Chi-square}_{(\text{calculated})} = 17.31$ , see Table S3).

**Table 2.** Numbers of sampled sites (Sites), numbers of species (NuSp), species per site (Sp/site), numbers of individuals (NuInd), species with (wst) or without (wost) natatory setae, and species with (wM) or /without (woM) males at 11 different elevational ranges with 100 m interval.

Elevational ranges m a.s.l.	Sites	NuSp	Sp/site	NuInd	wst	wost	wM	woM
0–100	23	21	0.91	2864	10	9	13	6
101–200	9	8	0.89	1056	3	5	4	4
201–300	13	7	0.54	2046	3	4	4	3
301–400	6	3	0.50	835	2	1	1	2
401–500	8	7	0.88	705	3	4	2	5
501–600	8	6	0.75	362	2	4	3	3
601–700	3	1	0.33	4	1	0	1	0
701–800	4	3	0.75	70	1	3	2	1
801–900	5	7	1.40	1169	5	2	5	2
901–1000	1	1	1.00	480	1	0	1	0
1001–1100 (1133)	3	3	1.00	7	1	2	2	1

Therefore, occurrence of natatory setae, presence of bisexual population and elevation are all mutually independent to each other, implying no direct effect of elevation on species distribution

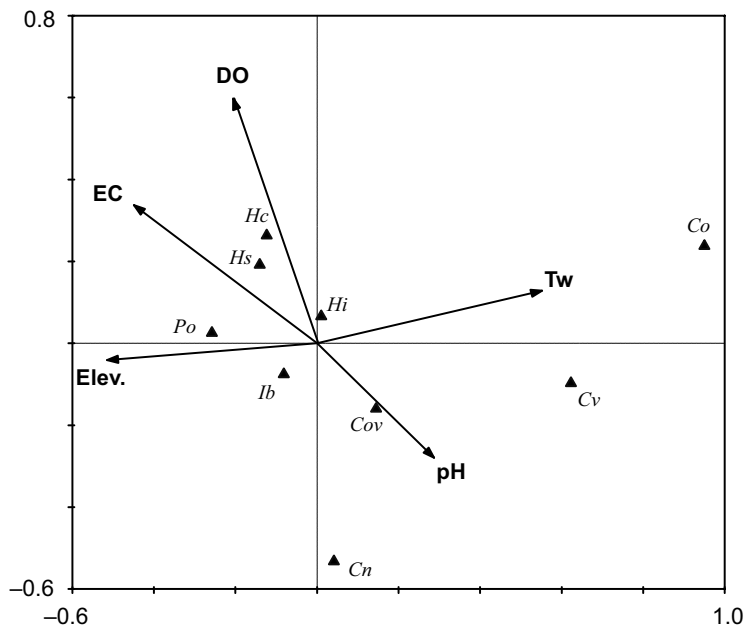
The first two axes of CCA explained 74.40% of relationships between species and environmental variables with a variance of 6.10 (Table S5).

The used environmental variables (electrical conductivity (EC,  $P = 0.38$ ,  $F = 0.87$ ), water temperature (Tw,  $p = 0.43$ ,  $F = 0.93$ ), elevation (Elev.,  $p = 0.55$ ,  $F = 0.88$ ), pH ( $p = 0.85$ ,  $F = 0.40$ ) and dissolved oxygen concentrations (DO,  $p = 0.72$ ,  $F = 0.71$ ) did not show significant effect on the distribution of ostracod species in Sakarya province. Four species (*Herpetocypris chevreuxi*, *Heterocypris salina*, *Psychrodromus olivaceus* and *Ilyocypris bradyi*) were situated on the left site of first axis where there are DO, EC and Elev. Remaining species (*Heterocypris incongruens*, *Cypria ophthalmica*, *Cypridopsis vidua*, *Cyclocypris ovum*, and *Candona neglecta*) were located at the site of Tw and pH and on the right site of axis 1. Of this species, *C. ovum* showed a close relationships with pH when *H. incongruens* was relatively closer to the center of diagram (Fig. 2).

As seen in Table 3, especially cosmopolitan species (e.g., *H. incongruens*, *I. bradyi*, *C. neglecta*) had higher tolerance levels when we compared with others except *C. ovum* for elevation. This means such species have a wide range of elevations. However, *Cypria ophthalmica* had lowest tolerance to elevation (17.76) and water temperature (0.65). Among the species *H. chevreuxi* had highest optimum value (6.87) while *C. neglecta* had lowest tolerance value for dissolved

oxygen concentrations (Table 3). These optimum and tolerance levels of species to different ecological variables determine the occurrence of them in any kind of habitats and geographical regions.

There was a strong negative correlation between *C. vidua* and EC ( $r_s = -0.88$ ,  $n = 6$ ,  $p < 0.05$ ) when a strong positive correlation between *P. olivaceus* and Elev. ( $r_s = 0.88$ ,  $n = 7$ ,  $p < 0.01$ ). On the other hand, the remaining species did not show any significant correlations with the used environmental variables. The correlations between environmental variables are like that pH-EC ( $r_s = -0.60$ ,  $n = 58$ ,  $p < 0.01$ ), DO-Tw ( $r_s = -0.49$ ,  $n = 58$ ,  $p < 0.01$ ), DO-Elev. ( $r_s = -0.54$ ,  $n = 58$ ,  $p < 0.01$ ) and Tw-Elev ( $r_s = -0.71$ ,  $n = 58$ ,  $p < 0.01$ ). In addition, the equation provided by trendline and R-square for the variables across elevational ranges are like for pH:  $y = 0.0242x + 8.0658$   $R^2 = 0.1264$ ; dissolved oxygen:  $y = 0.3209x + 4.6155$   $R^2 = 0.6365$ ; water temperature:  $y = -0.4479x + 16.27$   $R^2 = 0.8528$ ; air temperature:  $y = -0.4472x + 21.974$   $R^2 = 0.3936$  and for electrical conductivity:  $y = -14.012x + 539.02$   $R^2 = 0.1405$ . It seems that water and air temperatures and electrical conductivity had a tendency to decrease with the increase in elevational ranges while pH and dissolved oxygen concentration had a propensity to ascend.



**Fig. 2.** Ordination of nine species according to the effect of electrical conductivity (EC), dissolved oxygen concentration (DO), elevation (Elev.), pH and water temperature (Tw) on CCA diagram. Abbreviations for species: *Candona neglecta* (Cn), *Cypria ophthalmica* (Co), *Cyclocypris ovum* (Cov), *Cypridopsis vidua* (Cv), *Heterocypris incongruens* (Hi), *H. salina* (Hs), *Herpetocypris chevreuxi* (Hc), *Ilyocypris bradyi* (Ib) and *Psychrodromus olivaceus* (Po)



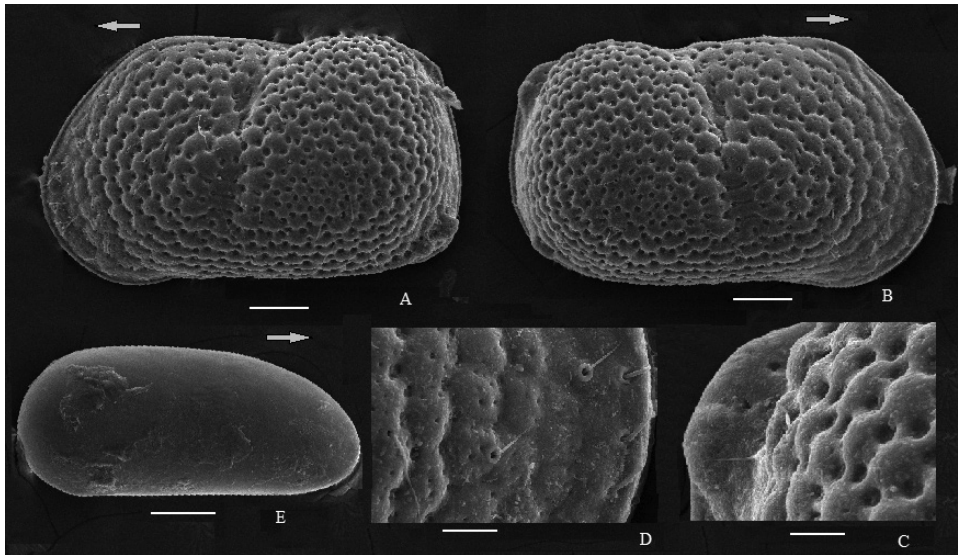
**Table 3.** Optimum ( $\mu_k$ ) and tolerance ( $t_k$ ) levels of nine species for pH, dissolved oxygen concentration (DO), electrical conductivity (EC), water temperature (Tw) and elevation (Elev.).  $N_2$  is the Hill's coefficient value that indicates the measure of effective number of occurrences.

Species	Count	Max	$N_2$	pH		DO		EC		Tw		Elev.	
				$\mu_k$	$t_k$	$\mu_k$	$t_k$	$\mu_k$	$t_k$	$\mu_k$	$t_k$	$\mu_k$	$t_k$
<i>H. incongruens</i>	35	792	8.55	7.72	0.83	6.35	1.67	697.65	301.03	14.53	1.88	379.82	365.76
<i>I. bradyi</i>	26	648	4.73	7.95	0.63	5.66	2.00	609.67	174.69	13.71	2.33	369.94	363.12
<i>P. olivaceus</i>	7	40	3.65	7.79	0.95	5.94	3.88	971.66	1076.36	15.50	6.34	517.61	225.32
<i>C. vidua</i>	6	12	3.30	8.36	0.19	4.85	1.78	320.06	155.19	16.51	1.66	56.44	182.09
<i>C. ophthalmica</i>	3	47	2.27	8.26	0.19	4.94	2.35	368.00	285.62	17.86	0.65	22.84	17.76
<i>C. ovum</i>	3	196	2.14	8.36	0.34	6.01	3.22	442.11	43.08	14.42	3.37	521.49	530.22
<i>C. neglecta</i>	9	38	2.09	8.41	0.43	4.41	0.79	422.58	247.53	14.32	1.72	457.53	311.63
<i>H. salina</i>	5	580	1.88	7.86	0.16	5.60	1.76	726.24	127.55	14.46	2.03	363.40	191.22
<i>H. chevreuxi</i>	4	360	1.28	7.85	0.40	6.87	1.61	836.65	256.46	14.10	1.30	262.17	157.58
			<b>min</b>	7.72	0.16	4.41	0.79	320.06	43.08	13.71	0.65	22.84	17.76
			<b>max</b>	8.41	0.95	6.87	3.88	971.66	1076.36	17.86	6.34	521.49	530.22
			<b>mean</b>	8.06	0.46	5.63	2.12	599.40	296.39	15.05	2.36	327.92	260.52

## DISCUSSION

Until now, 22 non-marine ostracod species have been known in Sakarya Province (ALTINSAÇLI 1997, GÜLEN & ALTINSAÇLI 1999, KILIÇ 2001). While six species (*Fabaeformiscandona fabaeformis*, *Cypris bispinosa*, *Cypris pubera*, *Dolerocypris sinensis*, *Eucypris lilljeborgi* and *Cyprideis torosa*) were not found in the current study, 16 of them were rediscovered from the area. Meanwhile the encountered 17 taxa were new reports for Sakarya Province (Table 1). Hereby, the number of ostracod taxa in Sakarya is increased to 39. Additionally, *V. cuneata* and *K. phreaticola* are new for Turkish Freshwater Ostracoda fauna (Fig. 3). Of which, *V. cuneata* is noted for the first time from the whole Palearctic region (MARTENS *et al.* 2013) and consequently, the geographical range of this species has been expanded with the present study (now in Afro-Tropical and Palearctic regions).

Canonical Correspondence Analysis results herein (Fig. 2) partially support the findings of KÜLKÖYLÜOĞLU *et al.* (2012b), YAVUZATMACA *et al.* (2015), KÜLKÖYLÜOĞLU *et al.* (2016) and KÜLKÖYLÜOĞLU *et al.* (2017a). In these previous studies, water temperature ( $T_w$ ), electrical conductivity (EC), pH, elevation (Elev.) and dissolved oxygen (DO) did not significantly effect the ostracod species composition. However, VAN DER MEEREN *et al.* (2010), KÜLKÖYLÜOĞLU



**Fig. 3.** SEM of *Kovalevskiella phreaticola* (A: external view of left valve, B: external view of right valve, C: protrusion on the postero-dorsal side of right valve and D: anterior part of right valve with pore openings and seta) and *Vestalenula cuneata* (E: right valve, external view). Scale is 50  $\mu\text{m}$  for A and B; 10  $\mu\text{m}$  for C and D; 100  $\mu\text{m}$  for E

*et al.* (2016) and COVIAGA *et al.* (2017) reported significant effect of pH, DO, EC, Tw and Elev. on species composition and distribution of ostracods. Additionally, MARTINEZ-GARCIA *et al.* (2015) noted ostracods species composition in Northern Spain were primarily effected by pH and EC when DO and elevation played secondary role. As pointed out above, insignificance effect of variables in the current study did not mean these variables do not have any effect on ostracod species composition in Sakarya province. Since, all of them have correlations between each other (see result section). Such kind of correlations may affect occurrences of species but we cannot completely estimate rate of the effect. Also, species show significant correlations (*C. vidua*-EC,  $p < 0.05$  and *P. olivaceus*-Elev,  $p < 0.01$ ) and they have different optimum and tolerances levels (Table 3) to these variables. Thereby, each variable has a direct or an indirect effect. This is because they are important variables determining the community in aquatic bodies.

Highest number of ostracod species (or taxa) were reported from low, mid and high elevations in literature (Table 4). However, the rejection of the first hypothesis showed that increasing of elevational ranges cause a decrease in the ostracod species number in the current study (Tables 2). This low ostracod species numbers may be explained as a consequences of rapid physical changes across altitudinal ranges. As one of the key factors of climatic changes, ambient temperature, determines the species richness of several terrestrial species at different elevations and an increase in elevation result as a linear decrease in temperature (McCain & Grytnes 2010). Recently, Castillo-Escrivà *et al.* (2016) also pinpointed that sites at high altitudes were wet and colder than the sites at low altitudes. The changes in air temperatures may eventually fluctuate the species richness and abundance of aquatic habitats (McCombie 1959, Kothandaraman & Evans 1972, Johnson *et al.* 2014). This is because of the linear relationships between the air and water temperature as previously reported (Schindler *et al.* 1990, Preud'homme & Stefan 1992, Reeves *et al.* 2007, Rogora *et al.* 2008). Our results were also found in support of these earlier findings as average water and air temperature decrease when elevational ranges ascend (Fig. 4). Any changes in water temperature can affect the physico-chemical variables of aquatic bodies (e.g., pH, dissolved oxygen, conductivity). Reeves *et al.* (2007) indicated the restriction of groundwater ostracods in the Pilbara region of northwestern Australia to mid-altitude locations since water depth, water chemistry, temperature and conductivity are altered by altitude. Külköylüoğlu *et al.* (2012d) and Rogora *et al.* (2008) showed the negative correlation between altitude and conductivity. Similarly, electrical conductivity herein showed a tendency to decrease with increasing elevations. Besides, occurrence of *H. salina* at low elevations (101–200 m, 301–400 m and 401–500 m, see Table S4) was also enforced the negative relation-

**Table 4.** The highest number of species or taxa (HNspT) from different elevational intervals or elevation (fEiE) compiled from literature. Abbreviation: The range of elevation (rE).

HNspT	fEiE	rE	Country	Citation
6 sp	1258	1258–2804	Mexico	PÉREZ <i>et al.</i> 2015
7 sp	336–607	335–944	Turkey	AKDEMİR <i>et al.</i> 2016
9 sp	166 and 901	5–1161	Patagonia	COVIAGA <i>et al.</i> 2017
10 sp	1170	759–2571	Mongolia	VAN DER MEEREN <i>et al.</i> 2010
12 sp	801–1000	0–1600	Turkey	KÜLKÖYLÜOĞLU <i>et al.</i> 2017b
13 sp	0–200	0–1400	Turkey	KÜLKÖYLÜOĞLU <i>et al.</i> 2017b
13 sp	1200–1400	0–1600	Turkey	KÜLKÖYLÜOĞLU <i>et al.</i> 2012c
15 sp	1231–1332	549–1457	Turkey	KÜLKÖYLÜOĞLU <i>et al.</i> 2016
20 sp	801–1000	432–1219	Turkey	KÜLKÖYLÜOĞLU <i>et al.</i> 2012b
21 T	652–752	450–1459	Turkey	YAVUZATMACA <i>et al.</i> 2015
26 sp	1650–1750	1659–2889	Turkey	KÜLKÖYLÜOĞLU <i>et al.</i> 2012a

ships between conductivity and elevations. Based on these reports, we may conclude that occurrence of ostracod species across elevational ranges seems to be related to their ecological tolerance to different variables. In the present study, cosmopolitan species or species with wide distribution and tolerance ranges (KÜLKÖYLÜOĞLU 2013) such as *H. incongruens*, *I. bradyi* and *C. neglecta* occurred in ten, nine and five of 11 elevational ranges, respectively (Table S4). The common occurrences of *H. incongruens* and *I. bradyi* was supported by the close location of them to the center of CCA diagram (Fig. 2), implying that they were not effected much by these variables while *C. neglecta* did not show any close relationships with ecological variables. As shown in Table 3, especially cosmopolitan species (e.g., *H. incongruens*, *C. neglecta*, *I. bradyi*, *P. olivaceus*, *H. salina*) have high tolerance levels when we compared with others. Having high tolerance levels allow them to adapt unsteady environment and contribute to their wide ranges for different variables (e.g., pH, dissolved oxygen, conductivity) they tolerate (KÜLKÖYLÜOĞLU *et al.* 2012a). For example, *P. olivaceus* and *C. ophthalmica* had highest (6.34) and lowest (0.65) tolerance levels for water temperature and their ranges (optimum  $\pm$  tolerance levels) are like that 9.16–21.84 and 17.21–18.51°C, respectively (Table 3). Therefore, cosmopolitan species are easily adapted to changeable conditions when compared with non-cosmopolitans. As a consequence, frequent occurrences of cosmopolitans decreased the rate of Beta diversity through elevational gradients and show the instability of environmental conditions.

The acceptance of second hypothesis indicates a regular distribution of number of species per site among elevational ranges. This hypothesis eliminates the effect of sampling sites across elevational ranges (Tables 2 and 4). Most recently, KÜLKÖYLÜOĞLU *et al.* (2017b) pinpointed that number of sam-

pling sites and number species per site did not show a linear relationship. This is also the case in the present study when the sampled sites equal to 23, 13 and 5, the species per site were 0.91, 0.54 and 1.40, respectively (Table 2). In other words, ascending in number of sampled sites did not increase the number of species per site encountered. This approach is actually the opposite one to "Sampling Effect Hypothesis" (HILL *et al.* 1994). Similar results were also reported previously (e.g., see KÜLKÖYLÜOĞLU (2004), NAGORSKAYA & KEYSER (2005), PIERI *et al.* (2009), AKDEMİR & KÜLKÖYLÜOĞLU (2011), YAVUZATMACA *et al.* (2015) and YAVUZATMACA *et al.* (2017)). On the other hand, KÜLKÖYLÜOĞLU *et al.* (2016) emphasized the significant relationships ( $p < 0.01$ ) between the frequency of occurrence and number of sampled sites in some elevational ranges. This is not the case in the current study, e.g., the most commonly encountered species herein *H. incongruens* showed occurrence frequency like 6, 7, 5 and 4 in 23, 13, 9 and 6 sampling sites, respectively. Other species also showed similar occurrence frequencies. As mentioned previously, occurrence and surviving of species in changing environments may depend on ecological conditions of habitats and tolerance abilities of inhabitants. As we tested in here, the number of species per site did not significantly different from each other from sea level to an 1133 m a.s.l. elevation. This is because the species with high tolerances (cosmopolitans) occurred almost in all of elevational intervals (see Table S4) that contribute to this regular results. This may be interpreted as that common occurrence of cosmopolitans may be indication of changing habitat conditions at different elevational ranges. If ostracods reach these types of habitats, species with cosmopolitan characteristics have advantage over non-cosmopolitans by means of having high ecological tolerance levels (KÜLKÖYLÜOĞLU 2013).

Due to the last hypothesis, occurrence of species with/without swimming setae and presence of sexual species are independent from the elevation. This result supports the findings of KÜLKÖYLÜOĞLU *et al.* (2012c) where the authors stated that reproductive modes of ostracod species was not significantly related to elevation. They also encountered with high number of asexual species (19 spp.) over sexual (11 spp.) from 0 to 1600 m a.s.l. when there is no differences in their occurrence frequencies across elevational ranges. Above 600 m (Table 2), however, the occurrence frequency of sexually reproducing species is higher than asexual when the general trend is toward to decrease in the number of species with/without males. In contrast to common occurrence of sexual forms over asexual, parthenogenetics are superior colonizers (HORNE & MARTENS 1999) by the reason of the introduction of one individual or egg in a habitat is enough to make a population but it requires two individuals of opposing sex in the sense of sexuals if they find to each other. This explain why the rate of natatory setae in sexual species is higher than asexual in the present study. This is because natatory setae of ostracods is used to actively

move in a habitat to find mate. Also, swimming or non swimming species were reported from all of the elevational ranges in the current study. A similar result was reported by KÜLKÖYLÜOĞLU *et al.* (2016). The above information enforce the active distribution of ostracods occur in the same habitat but passive distributions happens over geographical ranges. Thereby, one may consider the importance of species tolerance levels can be important issue when adaptive characters would be combined to increase the species survival chances. This may even be related to genetic variability of the species in future studies.

The importance of avian migration as an effective link between many different aquatic habitats and geographical regions was pinpointed previously. Besides elevational limits of avian species (ŞEKERCİOĞLU *et al.* 2008), human choose especially location near to sea level as residential areas. Hence, both vectors distributing the ostracods through long distances (GREEN *et al.* 2008, VALLS *et al.* 2016) can be limited by elevation. Along with the ecological preferences of ostracod species, limitation on passive distribution indicates the effect of elevation on ostracods. Consequently, elevation is a variable not to be ignored when talking about ostracods ecology and distribution. On the other hand, we should keep in mind that avian and human transportations are not the only way of passive dispersion.

In all, the results of the present study contribute to the non-marine ostracod fauna of Sakarya and Turkey. Cosmopolitan species occur frequently when elevation increase while species number may decrease. Due to this fact that species per site may not show differences among elevational gradient. As a result, the occurrence and distribution of ostracods across elevational gradients seem to be first depending on their ecological tolerance levels and later to their passive dispersion ability. Thereby, four common elevational patterns in species richness (McCain 2009) may be recognized for ostracods. They are i) decreasing richness patterns; a decline in species number with increasing elevation, ii) low plateau patterns; high species richness is at lower portion of the gradient but later decrease, iii) low plateau patterns with a mid-elevational peak; high diversity at low elevations when maximum species diversity occur above 300 m, and iv) low plateau patterns with mid-elevation peaks; species diversity peak at intermediate elevations. As a result, the distribution of cosmopolitans may not be limited by elevation but non-cosmopolitans distributions may be limited.

\*

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## SUPPLEMENTARY MATERIALS

**Table S1.** Two-tailed Kolmogorov-Smirnov Goodness of Fit test for vertical distribution of ostracod species ( $f_i$ ) from sea level to 1133 m a.s.l. Abbreviations and explanations:  $X_i$  (the last elevation of the range),  $f_i$  (observed frequency or number of species found at that elevation),  $F_i$  (sum of the observed frequencies from  $f_1$  up to end including  $f_i$ ),  $\text{rel } F_i$  (cumulative relative observed frequencies) =  $F_i/n$  ( $\sum f_i$ ),  $\text{rel } \hat{F}_i$  (cumulative relative expected frequency that is also refers to the distribution specified in the null hypothesis, e.g.,  $F_5 = 500/1133 = 0.44$ ),  $D_i$  (test statistics) =  $|\text{rel } F_i - \text{rel } \hat{F}_i|$ ,  $D'_i = |\text{rel } F_{i-1} - \text{rel } \hat{F}_i|$  and D is the

largest value of  $D_i$  or  $D'_i$ . If  $D > D_{\alpha,n}$  reject null hypothesis ( $H_0$ ).

$i$	$X_i$	$f_i$	$F_i$	$\text{rel } F_i$	$\text{rel } \hat{F}_i$	$D_i$	$D'_i$
1	100	21	21	0.31	0.09	0.23	0.09
2	200	8	29	0.43	0.18	0.26	0.06
3	300	7	36	0.54	0.26	0.27	0.04
4	400	3	39	0.58	0.35	0.23	0.08
5	500	7	46	0.69	0.44	0.25	0.07
6	600	6	52	0.78	0.53	0.25	0.07
7	700	1	53	0.79	0.62	0.17	0.14
8	800	3	56	0.84	0.71	0.13	0.18
9	900	7	63	0.94	0.79	0.15	0.17
10	1000	1	64	0.96	0.88	0.07	0.24
11	1100	3	67	1.00	0.97	0.03	0.28
					max	0.27	0.28

**Table S2.**  $\delta$ -corrected Kolmogorov-Smirnov Goodness of Fit test for vertical distribution of number of ostracod species per site ( $f_i$ ) from sea level to 1133 m a.s.l. If  $n \leq 25$ , the power of Kolmogorov-Smirnov testing can be increased impressively by employing;  $\text{rel } F_i = F_i / n + 1$  and  $\text{rel } F'_i = (F_i - 1) / (n - 1)$ .  $D_{0,i} = |\text{rel } F_i - \text{rel } \hat{F}_i|$  and  $D_{1,i} = |\text{rel } F'_i - \text{rel } \hat{F}_i|$ ,

the subscripts 0 and 1 are denoted  $\delta$  (lower case Greek delta).

$i$	$X_i$	$f_i$	$F_i$	$\text{rel }_i$	$\text{rel } \hat{F}_i$	$D_{0,i}$	$\text{rel } F'_i$	$D_{1,i}$
1	100	0.91	0.91	0.09	0.09	0.00	0.01	0.08
2	200	0.89	1.80	0.18	0.17	0.00	0.09	0.08
3	300	0.54	2.34	0.26	0.22	0.04	0.16	0.11
4	400	0.50	2.84	0.35	0.27	0.08	0.22	0.14
5	500	0.88	3.72	0.44	0.36	0.09	0.32	0.12
6	600	0.75	4.47	0.53	0.43	0.10	0.41	0.12
7	700	0.33	4.80	0.62	0.46	0.16	0.45	0.17
8	800	0.75	5.55	0.71	0.53	0.18	0.54	0.17
9	900	1.40	6.95	0.79	0.67	0.13	0.70	0.09
10	1000	1.00	7.95	0.88	0.76	0.12	0.82	0.06
11	1100	1.50	9.45	0.97	0.90	0.07	1.00	0.03
					max	0.18		0.17

**Table S3.** Mutual independence in  $11 \times 2 \times 2$  contingency table for species with/without developed natatory setae on A2 and males, and elevation. Degrees of freedom (df) = r (rows  $\Rightarrow$  elevational ranges) \* c (columns or sex (species with males) and asex (species without males)) \* t (tiers or layers  $\Rightarrow$  species with/without developed natatory setae) - r - c - t + 2 (for more see page 510 in Zar 1999) and so df =  $11 \times 2 \times 2 - 11 - 2 - 2 + 2 = 31$  herein. Expected frequency can be calculated by multiplying the total number of observations by the proportion of the total that the null hypothesis specifies for each category (see pages 467 and 511 in Zar, 1999), for example, Expected frequency of swimming species with sex at 0-100 m is  $(24/68) \times 19 = 6.71$ . Total Chi-Square ( $\chi^2$ ) calculated as  $\chi^2 = \frac{\sum \sum \sum (O - E)^2}{E}$ .

Elevation- al ranges	Observed frequency (O)						Expected frequency (E)						Calculated Chi-squares values									
	Swim			Non-swim			Swim			Non-swim			Swim			Non-swim			Total			
	sex	asex	sex	sex	asex	Total	sex	asex	sex	sex	asex	Total	sex	asex	sex	sex	asex	sex	asex	Total		
0-100	8	2	5	4	4	19	6.71	2.24	6.71	2.24	5.31	4.75	2.24	6.71	2.24	0.25	0.02	0.01	0.02	0.01	0.32	0.61
101-200	2	1	5	3	3	11	3.88	1.29	3.88	1.29	3.07	2.75	1.29	3.88	1.29	0.91	0.07	1.84	0.07	1.84	0.00	2.82
201-300	2	1	2	2	2	7	2.47	0.82	2.47	0.82	1.96	1.75	0.82	2.47	0.82	0.09	0.04	0.04	0.04	0.04	0.00	0.16
301-400	1	1	0	1	1	3	1.06	0.35	1.06	0.35	0.84	0.75	0.35	1.06	0.35	0.00	1.19	0.75	1.19	0.03	0.03	1.97
401-500	1	2	1	3	3	7	2.47	0.82	2.47	0.82	1.96	1.75	0.82	2.47	0.82	0.88	1.68	0.32	1.68	0.32	0.56	3.43
501-600	2	0	1	3	3	6	2.12	0.71	2.12	0.71	1.68	1.50	0.71	2.12	0.71	0.01	0.71	0.17	0.71	0.17	1.04	1.92
601-700	1	0	0	0	0	1	0.35	0.12	0.35	0.12	0.28	0.25	0.12	0.35	0.12	1.19	0.12	0.25	1.19	0.12	0.28	1.83
701-800	1	0	1	1	1	3	1.06	0.35	1.06	0.35	0.84	0.75	0.35	1.06	0.35	0.00	0.35	0.08	0.35	0.08	0.03	0.47
801-900	4	1	1	1	1	7	2.47	0.82	2.47	0.82	1.96	1.75	0.82	2.47	0.82	0.95	0.04	0.32	0.95	0.04	0.47	1.77
901-1000	1	0	0	0	0	1	0.35	0.12	0.35	0.12	0.28	0.25	0.12	0.35	0.12	1.19	0.12	0.25	1.19	0.12	0.28	1.83
1001-1100	1	0	1	1	1	3	1.06	0.35	1.06	0.35	0.84	0.75	0.35	1.06	0.35	0.00	0.35	0.08	0.35	0.08	0.03	0.47
Total	24	8	17	19	19	68															0.03	17.31

**Table S4.** Station types (Styp), ecological variables, and numbers of species (Code) occurred from each sampling site in Sakarya province. Sampling sites were ordered according to the elevational ranges with 100 m interval from sea level to 1133 m a.s.l. There is a blank row between ranges following to each other. Abbreviations: Station number (Stno), Station type (Styp), Station type (Styp), Dissolved oxygen concentration (DO, mg L<sup>-1</sup>), percent oxygen saturation (%DO), Electrical conductivity (EC, µS cm<sup>-1</sup>), Water temperature (Tw, °C), Air temperature (Ta, °C), Atmospheric pressure (AtmP, mmHg), Oxidation-reduction potential (ORP, mV) and Elevation (Elev, m a.s.l.). Coding (for taxa and species) is in Table 1.

Stno	Styp	pH	DO	%DO	EC	Tw	Ta	AtmP	ORP	Elev	Coordinate	Taxon code (# of individuals)
71	lake	8.86	3.73	40.8	464.1	19.1		761.3	78.3	0	N 41°05'19.1", E 030°44'74.0"	
64	lake	8.05	2.81	30	400.6	17.8		761.2	74.1	1	N 41°08'14.7", E 030°26'40.7"	Pk(96), Co(20), Cv(1), Cov(116)
54	lake	8.02	0.98	10.4	602	17.3		760.5	93.4	10	N 40°52'51.4", E 030°28'00.8"	Ck(5), Nm(3)
70	lake	8.70	3.49	37.1	418.7	18.2		761.2	79.7	11	N 41°01'89.1", E 030°33'46.9"	
55	lake	8.35	4.26	44.8	528	17.7		760.4	89.6	13	N 40°32'17.0", E 030°24'26.1"	Pk(292)
73	creek	8.46	4.36	42.7	303.7	14.1		758.9	81.3	18	N 40°59'60.5", E 030°56'02.6"	Ib(4)
57	trough	7.84	5.02	51	705	16.1		760	96.7	24	N 40°55'26.4", E 030°24'21.5"	
50	creek	8.55	5.8	58.1	400	15.6		758.5	80.5	26	N 40°52'29.5", E 030°21'17.1"	Cv(7), Hi(1)
52	slough	8.16	1.07	10.9	318.7	15.7		759.5	89.7	26	N 40°51'81.8", E 030°30'83.1"	Hi(2)
56	slough	8.13	2.47	24.7	267.6	15.2		759.9	84.8	27	N 40°55'24.8", E 030°24'37.8"	Cn(1), Cv(5), Hi(4), Ig(6)
66	trough	7.56	3.5	35.5	1012	15.9		758.2	90.1	27	N 40°59'90.3", E 030°29'60.6"	Cn(1), Hi(48)
53	lake	8.38	4.93	51.8	232.6	17.6		759.2	83.5	28	N 40°50'02.4", E 030°28'00.4"	Vc(3), Ps(14), Ph(11), Ck(117), Co(47), Cv(12), Mc(9)
51	trough	7.85	5.72	57.4	837	15.1		758.9	102.8	30	N 40°49'33.8", E 030°27'20.7"	Hi(188)
43	slough	8.17	8.52	92.9	844	19		756	76.6	39	N 40°41'62.2", E 030°16'45.5"	As(18), Ds(122), Co(12), Cv(1)
44	lake	8.54	8.45	88.6		17.7		756.8	63.5	39	N 40°41'62.2", E 030°16'45.5"	As(2)
62	slough	8.85	3.27	34.2	108.5	17.5		757.4	70.5	44	N 41°08'52.5", E 030°22'99.2"	
59	slough	7.93	4.12	41.1	656	15.3		757.7	94.1	50	N 41°00'80.6", E 030°17'51.4"	Cn(2), Ib(648), Im(72), Ige(6)
63	slough	8.28	3.34	35	249.3	17.3		757.5	41.2	51	N 41°08'94.3", E 030°23'39.7"	Ib(60), Im(11), Hc(14), Pa(24)

Table S4 (continued)

Stno	Styp	pH	DO	%DO	EC	Tw	Ta	AtmP	ORP	Elev	Coordinate	Taxon code (# of individuals)
69	trough	9.69	4.29	47.6	125	19.8		759.4	57.8	51	N 41°02'42.6", E 030°40'32.0"	Cn(1)
79	pond	8.23	4.18	40.6	339.7	13.8		756	90.2	58	N 40°44'71.5", E 030°43'87.7"	
67	spring	7.93	3.55	34.2	510	13.6		753.3	90.1	81	N 40°58'64.7", E 030°35'71.1"	Ib(16), Kp(50)
61	trough	8.83	3.86	38.1	339.4	14.68		754.1	83.3	84	N 41°07'07.2", E 030°19'84.7"	
49	trough	7.93	6.35	64.5	1047	15.7		752.9	92.5	87	N 40°49'44.1", E 030°14'72.1"	Hi(792)
39	creek	7.78	9.13	90.2	171.3	13	27	749.9	51.03	103	N 40°40'11.3", E 030°19'57.3"	Ib(1)
58	trough	8.04	4.97	49.1	599	14.2		752.7	95.3	104	N 40°57'75.4", E 030°18'87.1"	Hs(2), Ib(2)
65	slough	8.11	2.94	31.1	542	17.8		748.8	84.2	141	N 41°02'82.8", E 030°29'40.5"	
24	trough	7.96	5.23	52.2	620	15.2	18.7	746.3	82.8	148	N 40°27'34.3", E 029°59'89.8"	Hi(320), Po(4)
42	creek	8.69	8.66	82.5	206.2	12.9		747.8	65.5	161	N 40°40'36.3", E 030°14'73.2"	Pz(1), Hi(8), Ib(12), Po(4)
72	trough	8.65	4.01	40.2	136.6	15.3		746.1	71.2	169	N 41°01'09.7", E 030°52'97.2"	Hi(60), Pv(42)
48	trough	7.96	7.2	7.4	853	16.5		743.7	88.7	173	N 40°47'86.6", E 030°13'87.6"	Hi(200), Hs(280), Ib(8)
60	slough	8.00	3.76	37	710	14.6		758.4	97.6	194	N 41°01'69.3", E 030°19'95.8"	Cn(10), Ib(58), Ir(14)
34	slough	7.75	4.01	38.8	592	13.5	23	742	76.7	198	N 40°35'24.7", E 030°18'99.8"	Hi(1), Ib(24), Po(5)
25	trough	6.47	6.42	64.5	298.9	15.7	18.6	741.1	73.4	206	N 40°28'37.8", E 030°01'22.9"	Hi(112)
17	trough	7.98	7.96	78.2	659	14.1	20.1	738.2	49.7	221	N 40°29'12.2", E 030°18'32.5"	Hi(6)
37	slough	8.12	5.03	49.8	725	14.8	23	739.4	58.3	221	N 40°39'67.6", E 030°19'42.7"	Ev(18), Hi(1)
38	trough	5.58	6.05	60.9	295.2	15.7	23	739.4	51.3	221	N 40°39'67.6", E 030°19'42.5"	
77	slough	8.22	3.52	34.5	288.5	13.8		741.4	93.3	231	N 40°44'50.0", E 030°46'40.3"	Hi(7), Ib(2)
12	slough	8.22	7.23	73.9	483.5	15.9	19.4	735.2	72.8	240	N 40°30'94.8", E 030°20'84.2"	Hi(4)
13	trough	8.30	8.73	87.5	413.6	15.2	19.4	735.4	72.8	240	N 40°30'94.8", E 030°20'84.2"	Hr(1296), Ib(18)
36	trough	8.22	4.38	44.6	550	16.2	24	742.9	75.6	240	N 40°35'28.5", E 030°20'16.2"	
47	spring	8.12	5.09	53.4	555	17.4		737.5	83	242	N 40°46'88.2", E 030°15'40.6"	Cn(2), Hi(43), Ib(29)
45	trough	8.01	7.24	72.1	561	14.6		739.2	84.2	243	N 40°46'81.3", E 030°16'30.1"	

Table S4 (continued)

Stno	Styp	pH	DO	%DO	EC	Tw	Ta	AtmP	ORP	Elev	Coordinate	Taxon code (# of individuals)
68	trough	8.75	4.84	50	221.6	16.7		740.6	76.2	244	N 40°58'73.0", E 030°43'20.2"	Hi(108), Ib(4)
46	trough	7.78	6.89	66.8	865	14		737	89.8	250	N 40°46'89.7", E 030°15'85.0"	Hc(360)
74	trough	8.35	4.27	42.3	391	14.7		738.1	82.9	263	N 40°57'95.3", E 030°53'27.1"	Hi(2), Ib(34)
33	slough	6.17	4.73	47	745	15	24	731.1	74.9	322	N 40°34'97.5", E 030°17'91.7"	Hi(580), Ib(142)
21	pond	8.71	7.61	75.9	321.8	14.8	17.7	7.36	37.6	325	N 40°25'17.6", E 030°16'02.4"	
80	slough	8.77	4.05	41.3	210.3	15.9		731.5	85.4	338	N 40°39'49.1", E 030°40'68.1"	Hi(89)
83	trough	8.25	4.82	47.6	260.7	14.3		730.3	79	347	N 40°33'65.1", E 030°48'98.2"	Hi(8)
81	creek	8.80	5.76	53.3	190.2	11.6		727.4	64.2	389	N 40°36'89.7", E 030°32'12.8"	
11	trough	8.03	7.46	7.93	707.9	13.9	13.8	721.1	78.9	397	N 40°29'10.1", E 030°21'23.5"	Hi(4), Hs(11), Ib(1)
40	creek	8.57	3.62	34.9	162.3	12.7		724.6	55	406	N 40°39'94.7", E 030°17'11.2"	
15	spring	6.49	1.06	12.3	2488	24.3	15.9	722.2	53.5	407	N 40°28'31.8", E 030°21'28.5"	Hi(1), Ib(3), Po(25)
41	trough	9.11	6.34	61.7	109.1	14		723	50.4	421	N 40°39'85.5", E 030°16'96.8"	Hi(6)
35	spring	7.93	4.1	36.9	428.2	10.8	26	720.7	75	444	N 40°35'60.9", E 030°20'98.2"	Ds(1), Po(6)
16	pool	8.27	7.62	7.95	966	13.6	17.1	717.3	44	449	N 40°28'47.5", E 030°21'50.8"	Hc(19)
26	trough	7.79	4.74	46.3	669	13.5	18.3	719.4	62.6	453	N 40°29'19.0", E 030°01'28.2"	Hs(580)
1	slough	7.30	5.09	47.7	222.5	12.7	19.3	714	62.6	468	N 40°23'84.5", E 030°33'11.9"	Hi(2)
32	trough	8.50	8.6	83.2	559	14.1	20.5	715.4	73.6	499	N 40°32'91.3", E 030°10'89.6"	Hi(2), Hs(11), Ib(4), Po(10), Hc(16), Pfa(19)
18	trough	8.20	8.79	83.4	432.5	12.9	19.6	711.8	49.6	509	N 40°28'51.8", E 030°19'27.8"	Cov(23)
2	pool	7.69	7.48	72	443.4	13.4	20.3	710.6	73.5	520	N 40°25'63.2", E 030°33'74.5"	Pz(1), Ib(1)
3	trough	7.87	6.9	67.9	402.8	14	20.3	710.7	73.6	520	N 40°25'63.2", E 030°33'74.5"	Hi(1)
78	creek	8.51	4.81	44.8	131.5	11.4		714.3	81.7	544	N 40°44'01.9", E 030°50'04.1"	Pf(7)
82	slough	8.55	4.54	44.6	335.4	14		710.4	72.3	559	N 40°34'13.3", E 030°40'01.3"	Cn(38)
14	trough	8.30	9.5	8.79	539	13	20.9	707.2	74.1	564	N 40°27'76.0", E 030°21'67.9"	Hi(1)



Table S4 (continued)

Stno	Styp	pH	DO	%DO	EC	Tw	Ta	AtmP	ORP	Elev	Coordinate	Taxon code (# of individuals)
23	slough	8.40	8.1	73.1	438.8	10.8	17	707.2	50.6	569	N 40°26'43.2", E 030°10'76.6"	Ib(290)
76	trough	8.50	3.7	36.8	98.8	14.7		711.9	68.8	569	N 40°50'49.9", E 030°44'44.7"	
20	pond	8.02	6.21	58.3	619	11.2	20.4	700.2	60.6	635	N 40°26'59.3", E 030°17'61.0"	
22	trough	8.80	10.58	103.2	259.9	13.1	20.6	700.6	44.8	643	N 40°24'42.2" - E 030°10'75.1"	Hi(4)
27	creek	8.43	8.52	79.3	360.1	11.7	14	698.3	70.3	691	N 40°29'97.1", E 030°00'91.5"	
28	pond	8.67	7.1	71.6	232.9	14.8	12	693.1	67.4	702	N 40°31'42.5", E 030°00'69.4"	
19	trough	8.30	8.64	79.4	367.8	11.6	17.6	694.5	53.7	715	N 40°27'76.5", E 030°19'50.9"	Hi(5), Po(40)
9	trough	7.81	7.85	76.6	1434	13.3	18.2	693.2	87.1	720	N 40°26'71.8", E 030°30'47.5"	Hi(24), Ib(1)
29	slough	8.58	5.87	55	232.6	11.9	12	690.1	71.1	798	N 40°31'86.4", E 030°03'72.1"	
10	trough	8.44	9.96	99.2	567	13.7	19	686.3	76.2	810	N 40°26'85.3", E 030°57'40.3"	Hi(128), Ib(5)
75	slough	9.12	3.81	38.3	22.2	15.4		690.6	52.5	819	N 40°55'14.0", E 030° 49' 35.4"	
31	pond	8.57	7.58	800.8	467.8	12.6	19.2	700.5	74.3	831	N 40°32'33.6", E 030°04'57.4"	Ca(5), Cv(1), Cov(196), Ib(46), Np(8)
30	trough	8.09	7.15	65.8	707	11.7	18	696.1	82.7	833	N 40°32'33.6", E 030°04'57.4"	Hi(250), Ib(400), Pv(34)
4	trough	8.84	9.57	88.9	8.4	11.9	18.5	678.9	56.2	898	N 40°28'23.2", E 030°33'91.4"	Hi(96)
8	trough	7.95	8.32	78.2	514	11.4	20	671.6	83.1	998	N 40°28'09.2", E 030°31'88.8"	Hi(480)
5	pool	8.52	5.22	49.3	146.7	12.6	15.1	662.3	67.4	1099	N 40°29'69.5", E 030°33'88.6"	Cn(1), Ev(3), Ib(2)
6	trough	8.19	7.08	61.7	268.7	9.2	17.4	662	79.6	1100	N 40°29'97.0", E 030°34'06.4"	Cn(1)
7	pond	8.34	8.14	78.8	282.6	13.4	18.8	659.8	75.8	1133	N 40°30'01.3", E 030°34'97.9"	

**Table S5.** Summary of Canonical Correspondence Analysis for nine species and five environmental variables from 58 sampling sites. \* indicates the result of Detrended Correspondence Analysis.

Axes	1	2	3	4	Total inertia
*Lengths of gradient	5.27	3.79	3.79	3.33	
Eigenvalues	0.16	0.10	0.05	0.03	4.34
Species-environment correlations	0.46	0.41	0.28	0.28	
Cumulative percentage variance					
of species data	3.70	6.10	7.10	7.90	
of species-environment relation	45.60	74.40	87.60	97.30	
Sum of all eigenvalues					4.34
Sum of all canonical eigenvalues					0.35