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Research Article

Determining effective environmental factors and ecology of non-marine *Ostracoda* (*Crustacea*) in Giresun, Turkey

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Abstract – To determine influential environmental factors on ostracod species, 105 aquatic sampling sites were sampled from the Giresun province. Sixteen species collected from 69 sites are new records for the study area. Seven of 16 species were found in their expected geographical distribution while two species (*Ilyocypris bradyi, Psychrodromus olivaceus*) showed different distribution ($P < 0.05$). Of which, *P. olivaceus* displayed a limited distribution in the northern region of the study area. Geographical distribution of some species and their co-occurrences varied among habitats. The mean values of three variables (water temperature, electrical conductivity, and elevation) were significantly different in northern region than the values of the sampling sites in the southern region ($P < 0.01$). Canonical Correspondence Analysis explained 72.5% of the significant relationship ($P < 0.05$) between species and four most effective environmental variables (water temperature, electrical conductivity, elevation, and magnesium). *Heterocypris salina* and *Potamocypris fallax* exhibited maximum and minimum tolerance (and optimum) values for electrical conductivity, respectively. Heavy metal presence on the carapace surfaces was investigated using Energy Dispersive X-ray Analysis (EDX) along with SEM photographing. The observation of metals such as copper, aluminum, silver and even radioactive element such as technetium on the carapace surfaces suggests that the organisms studied actually carry much more information about their aquatic environment than it was thought. Overall, our results support the findings of previous studies that water temperature and electrical conductivity were the two most effective factors on ostracod species and can be responsible for their distribution and occurrences in sampling area.

Keywords: Ecological tolerance / effective factors / *ostracoda* / distribution / diversity

1 Introduction

Nonmarine ostracods can survive in almost all kinds of aquatic habitats (*e.g.*, springs, creeks, lakes, ponds, ditches, canals, reservoirs, peat moss and underground waters) from sea level to above 5000 m a.s.l. Also, several species are known from interstitial habitats. Among the 15 different freshwater taxonomic groups (*i.e.*, classes) of *Crustacea*, following *Copepoda* (Balian *et al.*, 2010), ostracods are the second-largest nonmarine group with about 2330 subjective species (Meisch *et al.*, 2019). By contrast to other crustaceans, ostracods have several other important biological and ecological characteristics including relatively short reproductive periods (*e.g.*, see Van Doninck *et al.*, 2003; Külköylüoğlu *et al.*, 2012c), large clutch size (Gandolfi *et al.*, 2001), short developmental stages (Cohen and Morin, 1990), high dispersal ability (passive or active dispersion) (Dole-Olivier *et al.*, 2001), presence of desiccation-resistant eggs (Horne, 1993), relatively high tolerance levels to different environmental variables (Akdemir *et al.*, 2016), and having both sexual and asexual (and/or mixed) populations. Besides, ostracods with their global distribution in a variety of freshwater and marine aquatic systems have very old fossil records going back to the Cambrian period (Williams *et al.*, 2008).

Freshwater ostracods are suitable bioindicators because they have a certain level of tolerance to some environmental variables (Benson, 1990; Delorme, 1991; Külköylüoğlu, 1999; Ruiz *et al.*, 2013). Studies have reported possible correlations between the occurrence patterns and distribution of the species with different environmental (*e.g.*, water temperature, salinity, dissolved oxygen) (Mezquita *et al.*, 2001; Wansard and Mezquita, 2001), biological (*e.g.*, presence of macrophytes, competition, and predation) (van Varten, 1983), and geographical variables (*e.g.*, elevation, temperature) (Horne and Boomer, 2000; Külköylüoğlu, 2005a; Pérez *et al.*, 2010).

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Ostracods are useful indicators for determining environmental changes (Wise, 1961; Ruiz *et al.*, 2004). Based upon the shape, size, and ornamentation, ostracods can be used as tools for describing past environmental conditions (Wise, 1961; Benson 1990). All ostracods possess two calcium carbonate valves (carapace) linked to each other medio-dorsally by a hinge structure. The carapace consists almost entirely of calcium carbonate contents (Turpen and Angel, 1971; Chivas *et al.*, 1986, but see discussion in Keyser and Walter, 2004). As a consequence, their calcitic valves readily preserve as fossils. Although Ca is a dominant element in the carapace, some other elements (*e.g.*, magnesium, strontium, sodium, phosphate, *etc.*) also occur in trace amounts. On the other hand, presence of trace elements on carapace is important issue because toxic effect of heavy metals can influence ostracod populations (Ruiz *et al.*, 2004; Khangarot and Das, 2009; Shuhaimi-Othman *et al.*, 2011). Species gain these elements directly from the aquatic environment they inhabit (Turpen and Angel, 1971; Palacios-Fest *et al.*1994; Wansard *et al.*, 1999). Since individual species represent the characteristics of waters in which they live, detection of those elements on carapace aids to understand characteristics of water quality, implying to understand past historical aquatic conditions. This refers to seek a possible relationship between carapace and chemical composition of the water which can be one of the influential factors on species occurrences.

Ostracods can be distributed evenly over long distances through passive or active transportation (Danielopol *et al.*, 1994). In passive mode, adults or eggs are transported by different factors such as wind, some plants, insects, amphibians, fishes, birds and humans (Horne *et al.*, 1998; Külköylüoğlu, 1999; Meisch, 2000, Rossi *et al.*, 2003; Valls *et al.*, 2016). The length of swimming setae can provide more efficient mobility of the ostracods active transport. Although ostracods have wide geographical distributional ranges along with many different habitat types, which environmental factor (s) can be a better predictor for their distribution is not well understood. Accordingly, the aims of the present study are to (i) contribute knowledge into the ostracod fauna of the Giresun province, (ii) assess the correlation between the most effective ecological variables on individual species along with calculating their ecological optimum and tolerance values, (iii) detect trace elements or heavy metals on the carapace, and (iv) observe distributional patterns of ostracods across the aquatic habitats of the province.

2 Materials and method

2.1 Area description

Giresun (40° 07′- 41° 08′ N 37° 50′- 39° 12′ E, 6934 km^2 in surface area) is in the Eastern Black Sea region of Turkey. The northern part of the area is bordered by 105 km of the Black Sea coast. At the same time, a fairly rugged Giresun Mountain range bound the south side (ca. 2000 m a.s.l.), which extend 50 to 60 kilometers from the shoreline of the sea. Two different types of climates characterize the area under the influence of the Giresun Mountains. One of them occurs in the northern part, marked as warm and with rainy conditions. The other with a continental climate is the Kelkit Basin at the southern part. The eastern Black Sea region has a relatively long rainy period. Hence, the lowest and highest temperature fluctuations varied from February (–9.8 °C) to October (37.30 °C) between 1929 and 2018, with total annual rainfall was of ~1288 mm/yr (107.33 mm/month) (Turkish State Meteorological Service, 2019). Karakaya *et al.* (2007) and Karakaya and Çelik Karakaya (2014) provide a detailed information on the chemical characteristics of more than 40 different aquatic systems (*i.e.*, spring, rivers, streams, and drainage waters) in the Giresun province (note that they sampled the area in 1996 and 1997, and their sites were different than ours sampled during the present study). According to their results about the water quality of the province, it was indicated that most of these water bodies were acidic (pH 1.85–8.53) in average of 5.20 with high sulfate content, and even in some cases, concentrations of Pb, Zn, Fe, and Cu was found in extreme levels (Karakaya *et al.*, 2007; Karakaya and Çelik Karakaya, 2014). Besides, the authors listed above also underlined that levels of some elements (*e.g.*, Cd, As, Mo, Se, Sb, Tl, Bi) occurred above the levels of acceptable (inter)national drinking water limits. Due to these high metal levels and pollution values, there can be a great environmental risk for life in these waters (Karakaya and Çelik Karakaya, 2014; pers. comm. to Karakaya, N). The waters studied by these authors are mostly acidic, low in dissolved oxygen (average 6.98 mg/L), and relatively cold waters (average 19.68 °C) with a high average value of EC (1838.3 mS/cm). Note that this high EC value might be due to the characteristics of the drainage systems (see Tab. 1 in Karakaya and Çelik Karakaya, 2014).

2.2 Sampling

In total, 105 water (Fig. 1) and sediment samples were randomly collected from different shallow (ca. 100 cm of depth) aquatic bodies during the period of 3–8 October 2015. Sampling included nine different aquatic habitats such as lake, creek, trough (man-made artificial container), ditch, stream, plunge pool of waterfall, river, pond and pool. Ostracod samples collected with a hand net (200 μm mesh size) from sediment (ca. 5 cm of depth) and open water were preserved in 70% ethanol in 250 ml plastic bottles. About 100 ml of water samples were also kept in clean plastic bottles for chemical analyses. All samples were saved at 4 °C in ice chests. Raw sediment samples were collected in Eppendorf vials for chemical analysis. After the field study, each ostracod sample was filtered with three-layer sieves (200, 150, 100 μm mesh size) in the laboratory under tap water and kept in 70% ethanol. We used needles to separate ostracods from sediment under a stereomicroscope (Olympus ACH 1X). Adult individuals were dissected in lactophenol solution for taxonomic identification. We followed the taxonomic keys of Meisch (2000) and Karanovic (2012) for species identification with the aid of a light microscope (Olympus BX-51). Using a JEOL JSM-6390LV Scanning Electron Microscope (SEM), photomicrographs of gold coated (4 μA) single valves were used. Valves werecoated them with a COXEM KIC IA model ION COATER. With the Energy Dispersive X-Ray Analyzer installed in the SEM, chemical composition of single valves of one individual per species was identified. All ostracod samples were stored at the Limnology Laboratory of Bolu Abant İzzet Baysal University, Turkey, and can be available upon request from the corresponding author.

Table 1. Comparison of the habitat types in the Giresun province with collected ostracods.

Habitat type	Total Sampling Site	Sites with Ostracod	Number of Species	Sites with ostracod (%)	Species per Sites
Lake	1	1	1	100.0	1
Creek	11	7	8	63.64	1.143
Trough	30	27	13	90.0	0.481
Stream	25	13	9	52.0	0.692
Pond	2	1	1	50.0	1
River	19	10	8	52.64	0.8
Ditch	7	4	4	57.14	1
Pool	4	2	2	50.0	1
Waterfall	6	4	5	66.67	1.25
Total	105	69			

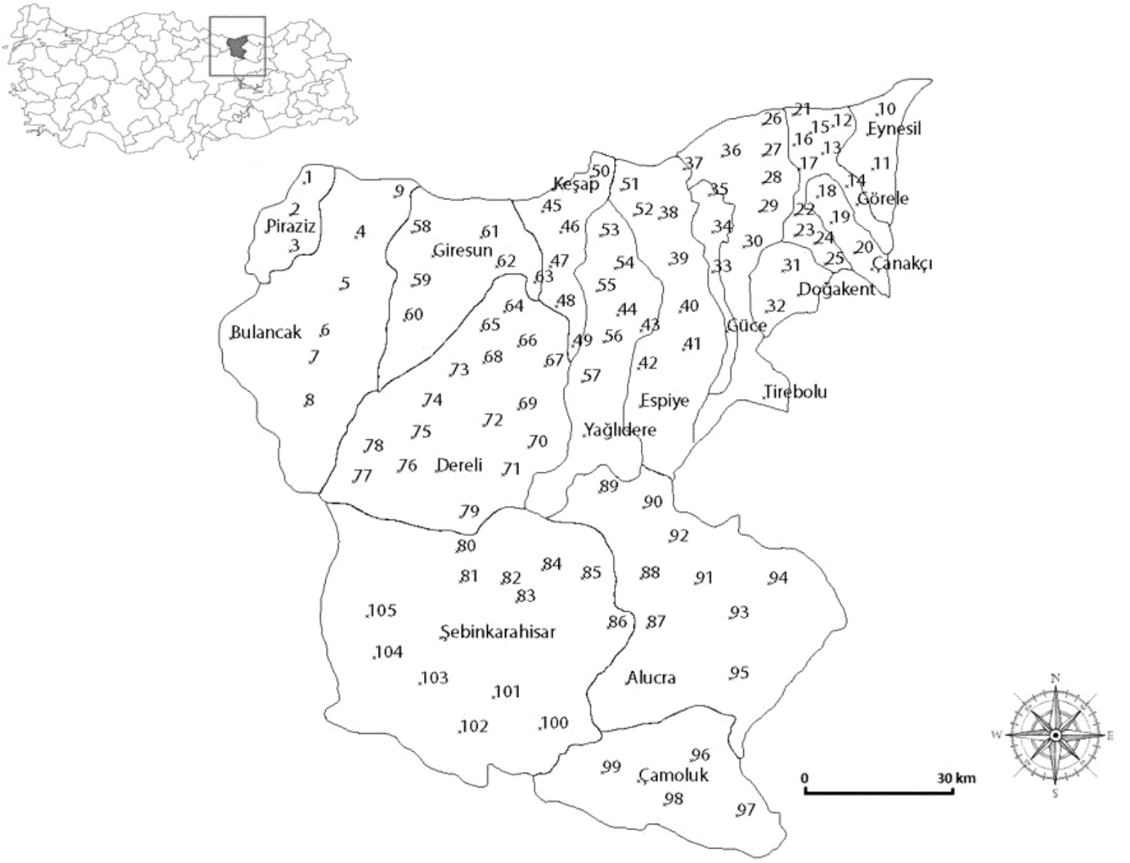

Fig. 1. Total of 105 randomly selected sampling sites (numbers shown on the map from 1 to 105) from 16 counties (Merkez, Alucra, Bulancak, Çamoluk, Çanakçı, Dereli, Doğakent, Espiye, Eynesil, Görele, Güce, Kesap, Piraziz, Sebinkarahisar, Tirebolu, Yağlıdere) of the Giresun province, Turkey.

In the field, we used a YSI-Professional Plus device to measure dissolved oxygen concentration (DO, mg/L), percent oxygen saturation (% sat.), water temperature (Tw, °C) electrical conductivity (EC, μS/cm), total dissolved solids (TDS, mg/L), salinity (Sal, ppt) pH, atmospheric pressure (mmHg). To obtain the meteorological data (wind speed (km/h), air moisture (%), air temperature (Ta, °C), at each sampling site, we used a Testo 410-2 anemometer. The site coordinates and elevation (m) were obtained using a Garmin e-Trex Vista H GPS. Furthermore, we analyzed water samples with the standard method (no: 4110) using Ion Chromatography (Dionex 1100) to calculate cations and anions of the sampled waters at the Department of Environmental Engineering, Bolu Abant İzzet Baysal University. Sodium (Na^{2+}), potassium (K^{2+}), magnesium (Mg^{2+}), calcium (Ca^{2+}), fluoride (F^{-}), chloride (Cl^{-}), nitrite (NO_2^{-}), nitrate (NO_3^{-}) and sulfate ($SO_4{}^{2-}$) characterized the water samples associated with organic and inorganic phosphate ($PO_4{}^{3-}$) from the sediments measured in parts per million (ppm).

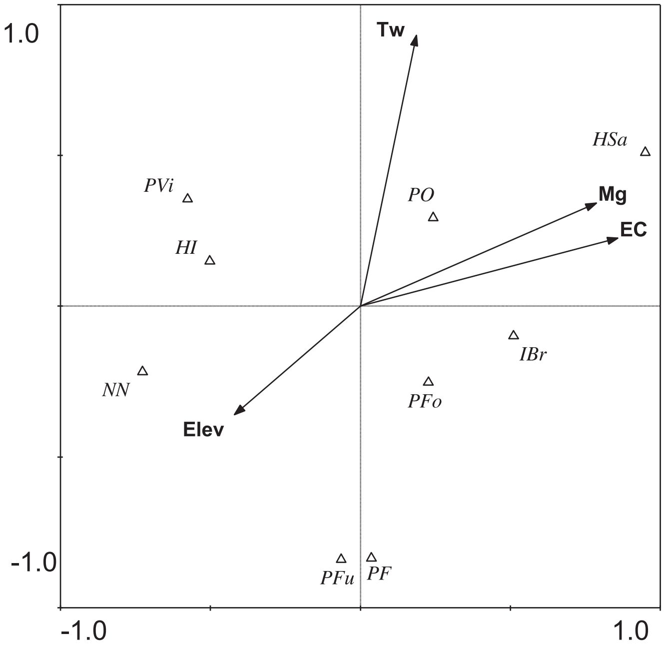

Fig. 2. The CCA diagram indicates the relationship between four ecological variables (water temperature (Tw), the magnesium content of water (Mg), elevation (Elev), electrical conductivity (EC)) and nine species (*Neglecandona neglecta* (NN), *Ht. incongruens* (HI), *Ht. salina* (HSa), *I. bradyi* (IBr), *Po. fallax* (PF), *Po. fulva* (PFu), *Psychrodromus olivaceus* (PO), *P. fontinalis* (PFo), *Po. villosa* (PVi)) from 64 different sampling sites in the Giresun province.

2.3 Statistical analyses

Using a Normality test, we calculated the ostracod species abundances (numbers of individuals per site). For this reason, we ran a one-sample Kolmogorov-Smirnov Z test for nine of the most frequently occurring species in the SPPS program (version 20.0). The C2 program calculated ecological optimum (uk) and tolerance (tk) levels of ostracod species encountered from three or more locations. Also, using the C2 software, we conducted the transfer function of weighted averaging regression (Juggins, 2003). Besides, as a multivariate statistical method, Canonical Correspondence Analysis (CCA) with a 499 Monte Carlo Permutation test was applied to detect possible relationships among ostracods and ecological variables. CCA was also used to portray the most effective environmental variable(s) on the ostracod species distributed among the sampling sites. The compatibility of the data for CCA was applied to log-transformation and then tested with DCA (Detrended Correspondence Analysis). Accordingly, values of DCA higher than three are convenient for CCA handling (ter Braak, 1987; Birks *et al.*, 1990). DCA values equal or greater than 3 are a sign that species fit the full Gaussian distribution. To minimize the arc-effect and eliminate the multicollinearity, we removed CCA values of species with rare occurrences and variables with a highly influential factor in the analyses. In the summary, larger eigenvalues of the first two axes offer a good explanation of the data (Birks *et al.*, 1990). The diagrams show the position of each variable and species analyzed across the sampling sites. It displays the correlations among the variables, species, and sites.

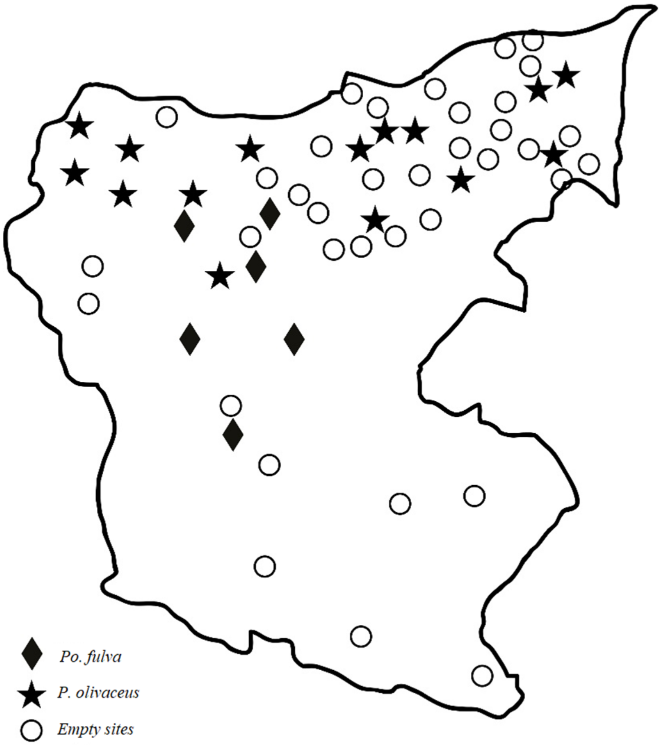

Fig. 3. Distribution of ostracod species (*Psychrodromus olivaceus, Potamocypris fulva*) in the Giresun province. Empty sites simply "absence of ostracods".

3 Results

A total of 16 ostracod species (*Neglecandona neglecta* (Sars, 1887)*, Cypridopsis vidua* (O.F. Müller, 1776)*, Herpetocypris reptans* (Baird, 1835)*, He. intermedia* Kaufmann, 1900*, Heterocypris incongruens* (Ramdohr, 1808)*, Ht. salina* (Brady, 1868)*, Ilyocypris bradyi* Sars, 1890*, I. brehmi* Schäfer, 1952*, I. inermis* Kaufmann, 1900*, Potamocypris fallax* Fox, 1967*, Po. fulva* (Brady, 1868)*, Po. villosa* (Jurine, 1820)*, Pseudocandona albicans* (Brady, 1864)*, Psychrodromus fontinalis* (Wolf, 1920)*, P. olivaceus* (Brady & Norman, 1889)*, Scottia pseudobrowniana* Kempf, 1971) occurred in 69 of 105 locations in the Giresun province. All species (Supplementary Material - Fig. S1) reported here are new records for the area. *Ilyocypris bradyi* was the most common species presented in 17 locations, followed by *P. fontinalis* (16 sites) and four other species (*P. olivaceus, Ht. incongruens Po. fallax, Po. villosa*) occurred in 15 sites (Fig. 2, Tab. S2). The most diverse sampling site was a trough with six species. Results indicated that the distribution of *P. olivaceus* was limited to the sampling sites distributed in the northern parts of the province (Fig. 3). The northeastern portion of the area of study did not contain ostracods, whereas the highest diversity occurred in the central and southern areas (Fig. 3). The two most common species found together from seven stations are *Ht. incongruens* and *Po. villosa*. During field work, we observed that the probability of finding a site without ostracods was somewhat 35% (69 sites with ostracods from 105 sites). In contrast, the ratio of finding ostracods was relatively high (90%) in the troughs (Tab. 1).

Table 2. CCA summary table with four variables (electrical conductivity, magnesium, nitrate and water temperature) and nine species with three or more times occurrences from the Giresun province (*DCA results).

Axes	1	2	3	4	Total inertia
Eigenvalues	0.297	0.210	0.156	0.036	4.266
Length of gradients*	4.072	4.057	3.790	3.273	
Species-environment correlations	0.640	0.577	0.498	0.243	
Cumulative percentage variance:					
of species data	7.0	11.9	15.5	16.4	
of species-environment relation	42.4	72.5	94.8	100.0	
Sum of all eigenvalues					4.266
Sum of all canonical eigenvalues					0.876

Table 3. Tolerance (Tol) and optimum (Opt) values for the nine most common species against the variables measured from each sampling site. Abbreviations: Count (numbers of species occurrence), Max (maximum numbers of individuals), N2 (Hill′s coefficient or measure of effective number of occurrences), dissolved oxygen (DO, mg l^{-1}), electrical conductivity (EC, µS cm^{-1}), water temperature (Tw, °C), redox potential (ORP), elevation (Elev), sodium (Na^{2+}, ppm) in water, magnesium (Mg^{2+}, ppm) in water, calcium (Ca^{2+}, ppm) in water, fluoride (F^{-}, ppm) in water, chloride (Cl^{-}, ppm) in water, total phosphate ($T.PO_4^{3-}$, ppm) in sediment.

				pH		DO		EC		Tw		ORP		Elev	
Name	Count	Max	N2	Opt	Tol	Opt	Tol	Opt	Tol	Opt	Tol	Opt	Tol	Opt	Tol
Neglecandona neglecta	4	9	2.4	7.3	0.8	7.3	1.1	238.61	561.55	11.3	3.6	369.72	570.55	1690.12	919.3
Heterocypris incongruens	15	225	4.7	8.1	0.3	8.3	1.8	540.86	510.08	12.9	1.3	344.22	316.56	1512.46	384.27
Ht. salina	11	190	4.3	8.1	0.3	7.7	0.8	2236.4	2152.9	15.5	2.5	359.87	31.19	792	430.01
Ilyocypris bradyi	18	135	3.4	8.0	0.3	8.1	1.3	1948.2	1265.5	13.1	1.9	361.32	637.8	1250.02	360.28
Potamocypris fallax	12	115	4.0	8.2	0.4	8.8	0.7	955.22	395.77	10.9	1.1	351.26	142.73	1364.8	265.69
Po. fulva	6	58	2.6	8.3	0.2	8.9	2.7	491.65	405.13	11.8	3.7	351.99	135.38	1250.78	863.19
Po. villosa	18	370	5.3	8.3	1	9.5	3.3	399.02	302.21	14	3.8	341.44	217.32	1468.9	543.7
Psychrodromus fontinalis	16	18	6.4	8.1	0.3	9	2.6	1208.7	1870.2	12.5	4.3	361.25	219.59	1249.12	732.83
P. olivaceus	12	49	2.5	8.4	0.3	8.4	1.4	857.31	234.74	17	2.5	345.56	164.81	335.11	332.09
Neglecandona neglecta	4	9	2.4	149	62	321	863	247.69	170.91	0.06	0.1	294.06	108.79	0.34	0.36
Heterocypris incongruens	15	225	4.7	897	61	395	517	380.5	235.39	0.07	0.3	283.93	166.49	0.63	0.33
Ht. salina	11	190	4.3	49	845	274	294	726.74	979.76	0.06	0.1	162.76	184.16	0.39	0.32
Ilyocypris bradyi	18	135	3.4	29	374	22	103	785.39	911.98	0.14	0.2	192.04	200.06	0.41	0.34
Potamocypris fallax	12	115	4	201	471	791	391	464.64	155.9	0.03	0	686.69	34.75	0.27	0.36
Po. fulva	6	58	2.6	373	275	602	698	248.19	225.84	0.02	0	221.87	42.91	0.76	0.23
Po. villosa	18	370	5.3	645	182	455	614	277.08	951.62	0.34	105	21.58	145.25	0.43	0.34
Psychrodromus fontinalis	16	18	6.4	394	806	156	291	312.41	247.84	0.02	0	897.65	150.16	0.46	0.38
P. olivaceus	12	49	2.5	128	570	954	511	471.65	218.39	0.08	0.1	568.68	888.62	0.58	0.24

Canonical Correspondence Analysis (CCA) explained 72.5% of variations between the relationships of nine species and four ecological variables (Tab. 2). CCA diagram (Fig. 2) showed that four variables (Tw ($F=3.044$, $P=0.003$), EC ($F=3.995$, $P=0.007$), Elev ($F=2.845$, $P=0.008$), Mg ($F=3.672$, $P=0.019$)) displayed significant correlation to the species while three other variables (Ca ($F=2.137$, $P=0.081$), DO ($F=1.095$, $P=0.336$), pH ($F=0.943$, $P=0.453$)) did not show significant relationships with them. Among the species, *N. neglecta* showed a high positive correlation with elevation but *Ht. Salina* displayed a positive correlation with Mg and EC. Individual species revealed different levels of tolerance and optimum values for different environmental variables (Tab. 3). Both *Ht. salina* and *Po. fallax* displayed maximum and minimum tolerance and optimum values for EC. By contrast, *N. neglecta* and *Po. villosa* had minimum optimum and tolerance levels for EC, respectively. The maximum tolerance level for water temperature was found for *P. fontinalis* when *Po. fallax* exhibited minimum tolerance and optimum estimates for the temperature. In contrast, *P. olivaceus* indicated the highest optimum value for Mg when *N. neglecta* has a maximum level of tolerance. Measured elements of the carapace by EDX revealed the presence of 15 different elements (O, C, Ca, Mg, Na, Sr, P, Cu, Rb, Al, Si, S, Tc, Ar, Ag) on the 17 ostracod valves (Tab. S1). As expected, three elements (O, C, and Ca) made up about 98% of the carapace structure (Tab. 4). Silver was found in the *P. olivaceus* valve while copper was found in the *Ht. incongruens* valve, and aluminum was in the *I. bradyi* and *C. vidua*. Moreover, as far as we know, this is the first time

Table 4. Elemental atomic percentage (%) values from the carapace surface of 17 ostracod species according to EDX analyses. Elements found in trace amounts are listed under 'Others'. Numbers written in parentheses indicate the number of sampling site. Tht sentence should continue with .... C (Carbon), O (Oxygen), Ca^{2+} (Calcium), Mg^{2+} (Magnesium), na^{2+} (Sodium), Sr (Strontium), P (Phosphate).

Species	C	O	Ca^{2+}	Mg^{2+}	Na^{2+}	Sr	P	Others
He. reptans (83)	36.05	48.88	14.05	0.20	0.30	0.03	0.49	
Ht. incongruens (14)	29.07	54.52	15.26	0.22	0.32	0.0	0.29	Cu
Po. fallax (72)	29.91	53.56	15.80	0.20	0.32	0.01	0.21	
Po. fulva (60)	27.50	53.72	17.96	0.25	0.30	0.01	0.26	
P. olivaceus (13)	31.11	52.38	15.40	0.41	0.33	0.0	0.30	Rb
I. brehmi (102)	23.70	57.38	17.86	0.32	0.34	0.13	0.28	
I. bradyi (71)	28.43	55.92	10.77	0.62	0.19	0.0	0.19	Al, Si
Ps. albicans (64)	26.77	54.82	17.54	0.17	0.27	0.04	0.25	S, Tc
Po. villosa (6)	30.57	52.97	15.62	0.19	0.28	0.01	0.35	
Ht. salina (60)	23.65	57.83	17.50	0.19	0.42	0.01	0.40	
I. inermis (74)	31.05	54.25	13.78	0.12	0.24	0.02	0.27	Rb
P. fontinalis (98)	27.59	52.06	19.38	0.36	0.30	0.05	0.26	
C. vidua (102)	33.28	49.88	15.63	0.28	0.37	0.0	0.38	Al, Si
*P. olivaceus (*24)	30.19	51.76	17.30	0.16	0.21	0.03	0.34	Ar, Ag
He. intermedia (102)	28.12	54.02	16.75	0.31	0.40	0.06	0.34	
Ht. incongruens (92)	24.15	59.12	16.19	0.08	0.25	0.01	0.19	Si
S. pseudobrowniana (15)	42.55	47.58	8.951	0.31	0.33	0.02	0.26	

Table 5. A) Mean, minimum (Min) and maximum (Max) values of environmental, and B) chemical variables measured from the sampling sites. Abbreviations: DO, dissolved oxygen; EC (electrical conductivity); Tw (water temperature); Atmp (atmospheric pressure); ORP (oxidation-reduction potential); elevation (Elev); Sal (salinity); TDS (total dissolved solids).

A)	pH	DO	EC	Tw	Atmp	ORP	Elev	Sal	TDS
Mean	8.19	8.50	985.97	14.62	694.29	341.81	771.95	0.61	771.30
Min	6.51	2.76	71.90	4.20	474.50	207.20	–8.00*	0.05	67.80
Max	9.96	15.82	15153.00	27.10	766.60	392.40	2752.00	8.40	9444.50
B)	Na^{2+}	Cl^{-}	Mg^{2+}	Ca^{2+}	NO_2^{-}	NO_3^{-}	SO_4^{2-}	$T.PO_4^{3-}$	
Mean	19.65	8.31	8.19	42.00	0.23	6.37	110.63	0.41	
Min	1.06	0.45	0.61	5.95	0.07	0.11	1.87	0.11	
Max	677.32	296.79	77.03	509.83	0.48	68.9	7462.2	0.95	

that trace amount of technetium (Tc) was encountered in the *Ps. albicans* valve. Our results following the known literature (41 ecology-related studies) revealed that water temperature and electrical conductivity (Tab. 5, Tab. S1) are the most critical factors responsible for ostracod species distribution and occurrences in the present study.

4 Discussion

4.1 Species occurrences and diversity

Previously, seven ostracod species (*Potamocypris steueri, Xestoleberis aurantia aurantia, Loxoconcha rhomboidea, Paradoxostoma guttatum, Callistocythere mediterranea, Pontocythere baceseoi, Eucytherura bulgarica*) had been reported from the Turkish province of Giresun (Kılıç, 2001). However, among those species, *Po. steueri* is the only one belonging to freshwater habitats. In contrast, the remaining species are known to inhabit brackish and/or marine waters along the Black Sea coasts. During the present study, *Po. steueri* did not occur in the area of study. To date, 17 species of nonmarine ostracods are known in the area, including *Po. steueri*. The diversity of species is low when compared to other parts of the region. For example, Erzincan (Akdemir and Külköylüoğlu, 2014) and Ordu (Külköylüoğlu *et al.*, 2012c) provinces to the south and west of Giresun include 32 (from 63 of 89 sites) and 26 (from 133 of 166 sites) ostracod species, respectively. This number is even lower than several other reports in other regions of Turkey such as 29 species in Gaziantep (Akdemir *et al.*, 2016), 22 species in Karabük (Külköylüoğlu *et al.*, 2017) and 22 species in Burdur provinces (Yavuzatmaca *et al.*, 2017a) and elsewhere, for instance, Italy (Pieri *et al.*, 2009). One may consider reasons for finding relatively fewer numbers of ostracods in Giresun. Before providing possible answers, it is important to point out that there were no significant differences in the methodology and sampling methods used in these studies. Besides, the number of sampling sites is also not the main problem since there are already other studies with less and more sampling sites (*e.g.*, see Rossi *et al.*, 2003; Martins *et al.*, 2010; Martínez-García *et al.*, 2015) than the present study. Accordingly, there can be at least three possible reasons for finding comparatively low numbers of species from Giresun. First, the timing of sampling (referring to month or season) may directly reflect on species occurrences. Most ostracods have a defined seasonal occurrence pattern (Lerner-Seggev, 1968; Rieradevall and Roca, 1995). It seems that both autumn and summer seasons in the northern hemisphere favor ostracods more than the spring and winter seasons (Külköylüoğlu, 1998; Akdemir, 2008; Gürer and Külköylüoğlu, 2019). We conducted fieldwork for a few days in the autumn; therefore, a comparison of seasonal differences cannot be considered herein with those of previous works. Indeed, there are other studies (*e.g.*, see Külköylüoğlu *et al.*, 2017, 2018) that sampled within the same month and/or same season where more species were reported. Second, there can be historical reasons for finding low numbers of species in the province. For this reason, palaeontological studies may provide an answer. However, at present, it is not possible to compare species numbers because there are no palaeontological studies in the area, whereas such studies are known for different regions of Turkey (*e.g.*, see Tunoğlu, 2003; Tuncer and Tunoğlu, 2015; Nazik *et al.*, 2018). Thirdly, the species characteristics, referring to competitive factors of dominant species over non-dominant ones, can be influential. In this case, eight (*N. neglecta, Po. villosa, Ht. incongruens, Ht. salina, C. vidua, Ps. albicans, I. bradyi, P. olivaceus*) of 16 species reported in the present study are thought to be cosmopolitan (Külköylüoğlu, 2013). Cosmopolitan do have some advantages over noncosmopolitan species. For example, they occur in a wide range of habitats (Mezquita *et al.*, 1999; Meisch, 2000; Martínez-García *et al.*, 2015; Yavuzatmaca, 2019) with wide ranges of tolerance and optimum values for different environmental variables (Külköylüoğlu *et al.*, 2013, 2016, 2018, 2019a, b). Cosmopolitan species have an important effect on species assemblages among the aquatic habitats. They use their selective advantages over noncosmopolitan species to reproduce seasonally and grow faster along with having high tolerance and optimum values for a/biotic factors (Smith and Horne, 2002). Eventually, these species can indeed be useful as beta diversity indicators (*e.g.*, see Nagorskaya and Keyser, 2005) between the areas where they can affect differences in total numbers of species (species diversity). Although we did not provide robust evidence for the effect of cosmopolitan species on total diversity, they were the species with the most frequent occurrences among the sites during the present study.

4.2 The effect of ecological factors on the distribution

Figure 2 portrays CCA results with four variables (Tw, EC, Elev, Mg) found with close relationships with ostracods from the Giresun province. This result coincides with the known studies available in the literature (Tab. S1) that water temperature and electrical conductivity were the two most influential variables controlling ostracods. Indeed, as stated above, out of 42 ecology-based studies, 29 and 22 of them underlined that water temperature and electrical conductivity were the most useful/effective variables, respectively. Besides, according to these studies, pH and elevation were only found valuable in 13 and 11 of them, respectively. However, the influence of these variables on individual species can show differences. For example, Figure 2 clearly illustrates a close correlation between *Ht. salina* and electrical conductivity and magnesium content (compare the arrows in Fig. 2). Still, some other species are on the opposite side of the arrows. Previous investigations (*e.g.*, Meisch, 2000; Fuhrmann, 2013; Scharf *et al.*, 2016) report *Ht. salina* from fresh to brackish waters where it can tolerate high levels of salinity values. Pint *et al.* (2015) clustered this species in waters with high contents of chlorides. In contrast, we found it from the waters with a high concentration of Ca and SO_4^{2-} with a relatively low (8.9 °C) to medium (19.7 °C) temperature ranges. However, it was collected in warm waters with year-round temperature of 26 °C (Külköylüoğlu *et al.*, 2013) or even up to 34 °C (Pax 1942, 1948).

Similar to *Ht. salina*, *Ilyocypris bradyi*, the most common species responding to a wide range of variables thrives in almost all types of aquatic bodies (Pieri *et al.*, 2009; Li *et al.*, 2010). The correlation between the two species was positive ($P < 0.05$). *Ilyocypris bradyi* is a non-swimmer benthic ostracod, but *Ht. salina* can swim. This may explain finding a positive correlation between the two species since *Ht. salina* can swim and change its location in a water body when *I. bradyi* prefers bottom. Differences in mobility and microhabitat preferences may eliminate competitive interaction between these two cosmoecious species (Külköylüoğlu, 2013). In contrast, *Ht. incongruens*, another well-known cosmoecious species (Külköylüoğlu, 2013) is on the opposite side on the CCA diagram. This species lives in a variety of waters with high ranges for salinity (3320 μS/cm) (Mezquita *et al.*, 1999), temperature (31.7 °C) (Külköylüoğlu, 2013), and low oxygen (1.0 mg/l) (Külköylüoğlu, 1999) levels (also see Yavuzatmaca and Külköylüoğlu, 2019). However, the fact that *Ht. incongruens* tolerates wide ranges of ecological variables amid shallow aquatic bodies with specific amounts of phosphate components (Pint *et al.*, 2015; Yavuzatmaca and Külköylüoğlu, 2019). Our study supports the hypothesis that the species has very high optimum levels for total phosphate. Similar to *Ht. incongruens*, two other cosmoecious species (*N. neglecta* and *Po. villosa*) occur on the left side of the diagram (Fig. 2). *Neglecandona neglecta* is closer to the arrow of elevation on the diagram while *Po. villosa* is far above it. These species are of similar cosmoecious characteristics which are widely distributed among different types of aquatic habitats where they are also known to tolerate a variety of aquatic conditions (Külköylüoğlu, 2013). Pint *et al.* (2015) did not find *Ht. incongruens* and *C. vidua* in waters with high Ca contents and underlined that their absence could indicate such waters in studies aiming to reconstruct past historical conditions. Although *C. vidua* was found from two different habitats during our research, in both cases, the species was found from Ca and SO_4^{2-}-dominated waters. Supporting evidence to these findings comes from the study of Peterson *et al.* (2013). They found live specimens of *C. vidua* from the Frasassi sulfidic spring adjacent to the Frasassi Caves system in northeastern Apennines of Italy. These results suggest that *C. vidua* has some levels of tolerance in waters enriched in sulfate contents. Several studies reported *C. vidua* from low (37 μs/cm) (Meisch and Broodbakker, 1993) to high levels of salinity (7410 μs/cm) (Meisch *et al.*, 2007) where it can tolerate low oxygen levels (Külköylüoğlu, 2003; Martins *et al.*, 2010).

Psychrodromus olivaceus thrives in freshwater habitats, including springs, creeks, ponds, troughs, and slightly flowing zones of streams. Similar to *C. vidua*, the species was initially encountered in Ca and SO_4^{2-}rich waters, but it can also be found in waters with medium Mg and Cl concentrations. For example, in a monthly study focusing on the association between the chemical structure of ostracod valves and the environmental variables, *P. olivaceus* accompanied with *Po. similis* was studied all year-round in a cold (water temperature 9.6–10.7 °C), slightly alkaline (pH 7.11–7.75) freshwater (EC 190.2–342 414 ms/cm) rheocrene spring in Turkey (Külköylüoğlu *et al.*, 2015).

Pseudocandona albicans is another cosmopolitan species, but we did not use the species in correlation analyses due to its single occurrence. Nevertheless, it is a benthic species that is mostly be encountered in the cold to warm springs, creeks, streams, and ponds (Meisch, 2000), as well as in the association of riparian habitats (Iglikowska and Namiotko, 2012). As shown above, although these eight individual species bear typical cosmopolitan characteristics, their levels of tolerance and optimum values vary among the habitats at different elevational ranges. We also found elevation (and temperature) to be one of the four essential variables on species distribution. However, previous studies have reported argumentative results about the effect of elevation/temperature. While some studies (*e.g.*, Mezquita *et al.*, 1999; Pieri *et al.*, 2009) argued that elevation is an influential factor on ostracods, other authors (Laprida *et al.*, 2006; Külköylüoğlu *et al.*, 2012c; Guo *et al.*, 2013; Yavuzatmaca *et al.*, 2015, 2018) did not support this view. However, current studies (see discussion in Yavuzatmaca *et al.*, 2018) indicated that elevation indirectly play as a secondary role in ostracod distribution and dispersion. Future studies are needed to find more solid evidence for the relationship between altitude and ostracod species distribution and occurrences.

Psychrodromus fontinalis, the second most common species with 16 sampling sites throughout the present study, is a common species with a high tolerance range for environmental factors. This is why it has been observed in six different habitat types. We do not know much about the 13 ecological characteristics of this species, but it is usually reported from springs, waters related to springs, and subterranean waters (Meisch 2000). Most recently (Külköylüoğlu *et al.*, 2020), *P. fontinalis* was reported from springs where the species showed highest optimum value for redox potential (opt = 82.17) and lowest values for pH (opt = 7.55) and water temperature (opt = 14.23). During the present study, the species thrived in a relatively cold creek (9.1 °C) and trough waters (17.8 °C) where average Ca (41.37 ppm), and Mg (33.01 ppm) values were also relatively high.

In comparison with the other species, *I. bradyi* has a "thick" and ornamented carapace and is known to tolerate a variety of ecological variables (Yavuzatmaca *et al.*, 2017a, b). For example, according to Bunbury and Gajewski (2005), *I. bradyi*, referring waters with high Mg/Ca contents, also showed high tolerance for the amount of sodium (ca. 12 mg/l) (see details in their Fig. 6) in the waters. Our study does not support Bunbury and Gajewski′s research, and we found that the species from a variety of waters showed a wide range of tolerances to calcium and water temperature (Tab. 3). The content of Na in *Ht. salina* and *He. intermedia* is greater than that of other species. Considering the information gained from the previous studies (Karakaya *et al.*, 2007; Karakaya and Çelik Karakaya, 2014), it can be assumed that most of the waters studied in the region are rich in SO_4^{2-}. As indicated above (Tab. 5), *Ht. salina* was found from Ca and SO_4^{2-}-dominated waters during this study. This occurrence may be a coincidence as shown by other species found in waters with different chemical contents. However, if this is true, it can be useful to apply it in palaeontological studies on fossil ostracod valves such as *Ht. salina* which has been frequently reported from different regions of Turkey (Tunoğlu, 2003; Tuncer and Tunoğlu, 2015; Nazik *et al.*, 2018). Thus, knowledge about carapace chemistry may aid to estimate past aquatic conditions (Ito *et al.*, 2003) since species prefer certain kinds of waters (Akdemir *et al.*, 2016).

Species, like *Ht. salina*, can usually be found as fossil due to their well calcified (*i.e.*, calcite crystals) carapaces (Keyser and Walter, 2004). Keyser and Walter (2004) indicate that organisms with a poorly calcified carapace do not produce crystallites. In such a case, they may not be found as fossils because the amorphous calcite is not strong enough in waters where they would dissolve. Weak calcification may result during the ecdysis (moulting) of the ostracods carapace (Keyser and Walter, 2004). Consequently, ostracods that inhabit waters with low calcium concentration or high salinity may not fossilize well (*e.g.*, not enough time for calcification, strong acidity, moulting stage, *etc.*).

Unlike *Ht. salina*, little ecological information is available for *He. intermedia* found only once in a trough during this study. The species is known from several different types of 14 aquatic habitats (*e.g.*, creek, spring, stream, pond, pool, littoral zones of lake and reservoir) (see details in Külköylüoğlu *et al.*, 2012d; Akdemir and Külköylüoğlu, 2014; Uçak *et al.*, 2014; Yavuzatmaca *et al.*, 2015). Water temperatures in these environments ranged from 13.9 to 32.3 °C, Ca (8.09–119.13 ppm), and Mg (5.54–48.20 ppm). Two earlier studies (Wansard *et al.*, 1999; Wansard and Mezquita, 2001) specifically focused on the correlation between water and carapace chemistry of this species. Wansard *et al.* (1999) collected *He. intermedia* from the River Magre (Valencia, Spain) with a broad water temperature range (5–20 °C) and with conductivity values less than 1 mS/cm, and the ratio of Mg/Ca of the water measured between 0.1 and 1. Wansard *et al.* (1999) reported that temperature was not an influential factor in the partition coefficient value of Mg (D(Mg)). During the present study, we measured water Ca and Mg levels as 51.54 and 20.41 ppm, respectively. The ratio of Mg/Ca of the water was about 0.396 ppm, similar to that of earlier studies. Besides, Wansard and Mezquita (2001) indicated that temperature was not an influential factor in the elements of the carapace. However, the opposite results calculated from their data were also found (see details in Dettman *et al.*, 2002). The implication of these results suggest that *He. intermedia* prefer slightly acidic (pH 6–6.04) (Külköylüoğlu *et al.*, 2012d; Yavuzatmaca *et al.*, 2015) to alkaline freshwater habitats (pH 7.2–8.5) (Wansard and Mezquita, 2001). Increasing salinity may decrease in the ratios of Sr/Ca and Mg/Ca (Wansard and Mezquita, 2001). Thereby, Ca is a dominant element in *He. intermedia* carapace that may explain its common occurrences in alkaline spring waters. Chivas *et al.* (1985) argued that Sr in the valves of all ostracods, within the same genus, is not affected by water temperature; therefore, Sr values in carapace may correspond to the Sr in waters. Except for two genera 15 (*Potamocypris* and *Heterocypris*), species of other genera did not support the view of Chivas *et al.* (1985).

4.3 Effectiveness of EDX in detecting heavy metal on the carapace surface

Anthropogenic impacts, especially industrial activities, create an intense disturbance on aquatic ecosystems (Alin *et al.*, 1999). One of the most important factors that cause this pollution is heavy metals. On the other hand, abundance and diversity of the ostracods can be affected by the metals in their surrounding water (Ruiz *et al.*, 2013). However, it has also been observed that some ostracod species are quite resistant to metal pollution such as lead (Prasuna *et al.*, 1996). Although it gives different results to different types of pollution, it is known that the diversity of ostracods is inversely proportional to pollution (Ruiz *et al.*, 2005). In short, it is one of the responsibilities of hydrobiologists to analyze both the amount of metal contamination of the aquatic habitat and whether the organism uptakes the metal.

The method tested in the present study is to apply EDX analysis by focusing the center of the carapace during SEM monitoring. The data displays the atomic and molar percentages of elements that belonging to the shell surface in a frame. Elements such as carbon, oxygen, calcium, magnesium are not useful since they do not give what compound they belong to. On the other hand, it only scans limited part of the surface and it is insufficient to generate a full range of carapace chemistry data. Since EDX scanning of samples prepared for SEM monitoring does not require any extra preparation or processing, it is thought that it can be functional in obtaining a quick preliminary idea about the presence of trace elements like heavy metals. In this regard, the heavy metals found in some of the 17 different sample scans made suggested that the method could yield efficient results when used for this purpose. Likewise, heavy metals do not give a clear result because there is only one sample belonging to the same habitat in the present study. In a hypothetical study where heavy metal traces were found consistently in a study with EDX analysis results with all SEM monitoring, it could provide evidence of heavy metal pollution in the studied habitat.

5 Conclusion

The species distribution observed in the field shows a high tolerance and optimum values for salinity and temperature. For example, *Ht. salina* and *I. bradyi* are widely spread over the region among a variety of habitats. An exception was *P. olivaceus*. While occurring in seven different habitat types (stream, river, creek, trough, waterfall, water body, and pool) along with the mountain ranges, *P. olivaceus* remains limited in the northern part of the Giresun province in good agreement with the species′ negative correlation to altitude (Külköylüoğlu *et al.*, 2013). Still, some other factors (*e.g.*, biotic factors, not discussed herein due to lack of biological data) can also affect its distribution. Similarly, *I. inermis* and *Po. fulva* wer found within the Dereli region (Fig. 1). *Psychrodromus olivaceus* thrives mainly in cold running waters and/or springs, spring-related streams, lakes, caves, and rice fields (Baltanás *et al.*, 1993; Meisch 2000). In some cases (*e.g.*, this study), the species exists in troughs. Comparative analyses between chemical contents of sampling sites located on southern and northern regions of the Giresun province revealed two factors (elevation and water temperature) were statistically significant differences. At the same time, pH, EC, and DO were not significant ($P > 0.05$). Since water temperature tends to decrease with increasing elevation, interpretation of these results appears to provide supportive evidence that these factors are highly influential on the distribution of *P. olivaceus*. Similar ideas would also be used to apply for other species (*e.g.*, *Po. fulva*) if there were enough amounts of ecological data available.

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Figure S1. SEM photographs of some ostracods in this study.
Table S1. Effective factors on the ostracod species determined by the present and previous studies.
Table S2. Numbers of stations and species found during this study from different habitat types in Giresun province.

The Supplementary Material is available at https://www.limn.org/10.1051/limn/2022002/olm.

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