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Reporting of Sexual Population of *Heterocypris incongruens* (Ramdohr, 1808) from a Man-Made Pond (Kahramanmaraş, Turkey) and Comparison of Hemipenes of the Genus *Heterocypris* Claus, 1892

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Research Article

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Abstract

The current numbers of the genus *Heterocypris* includes about 68 species. When 37 species have males, males of 26 species are not known and data is not available for five species. Among the species of the genus, *Heterocypris incongruens* has a cosmopolitan distribution but the males of the species have only been reported in Palearctic and Nearctic regions. During an extensive field sampling, total of 6 males and 59 females were collected from Haydolar pond (a man-made pond) in Pazarcık, Kahramanmaraş (Turkey) on 11 June and 08 September of 2010. During this study, we focused on the description of the hemipenis and compared it with others in the genus. Sexual ratio is about 1:10 in favor of females. Zenker's organ has 28 whorls. The lenght of the hemipenis (Lh =0.23 mm) was about 19% (ca 1/5) of the length of the carapace (L: 1.21 mm) in our samples. Distal and proximal shields of the hemipenis show variability within the indivudals of the male *H. incongruens* but these shields are similar among some other species (e.g., *H. punctata* and *H. putei*). Such similarities in hemipenes may support the fact that the species of the genus may have a monophyletic group. Accordingly, our study suggest that using hemipenis as a taxonomic character should be done with great attention because the shape of hemipenis can be changed by means of several factors.

Keywords: Heterocypris, hemipenis, Kahramanmaraş, monophyletic, Palearctic

***Heterocypris incongruens* (Ramdohr, 1808)'in seksüel popülasyonunun insan yapımı bir gölette (Kahramanmaraş, Türkiye) rapor edilmesi ve *Heterocypris* Claus, 1892 cinsinin hemipenislerinin karşılaştırılması**

Özet

Heterocypris cinsine ait 68 türün 37 tanesinin erkeği bilinirken 26 türün ise erkeği bilinmemektedir ve beş türün erkeğinin olup olmadığı hakkında ise bilgi bulunmamaktadır. *Heterocypris incongruens*, cinsin türleri arasında kozmopolit bir dağılıma sahiptir, ancak türün erkekleri sadece Palearktik ve Nearktik bölgelerde rapor edilmiştir. 11 Haziran ve 8 Eylül 2010 tarihlerinde Pazarcık (Kahramanmaraş, Türkiye)'taki Haydolar göletinde yapılan kapsamlı bir arazi çalışma sırasında toplam 6 erkek ve 59 dişi birey toplanmıştır. Çalışma sırasında, hemipenis'in tanımına odaklandık ve bunu cins içerisindeki diğer hemipenisler ile karşılaştırdık. Seksüel oranı yaklaşık 1:10 değeriyle dişiler lehine olduğu tespit edilmiştir. Zenker organında 28 tane sarmal vardır. Örneklerimizdeki hemipenis'in uzunluğu (Lh = 0.23 mm), karapaks (L: 1.21 mm) uzunluğunun yaklaşık %19'u (yaklaşık 1/5) kadardır. Hemipenis'in distal ve proksimal kısımları, erkek *H. incongruens* bireyleri içinde değişkenlik gösterirken bu kısımlar diğer bazı türler arasında benzerlik göstermektedir (örn., *H. punctata* ve *H. putei*). Hemipenisler deki bu benzerlikler, cinsin türlerinin monofiletik bir grup olabileceği gerçeğine bir destek olabilir. Buna göre, çalışmamız hemipenisin taksonomik bir karakter olarak kullanılmasının büyük bir dikkat gerektirdiğini çünkü hemipenisin şeklinin farklı faktörlerle değişebileceğini önermektedir.

Anahtar kelimeler: Heterocypris, hemipenis, Kahramanmaraş, monofiletik, Palearktik

INTRODUCTION

The class Ostracoda is a wide-spread group of small crustaceans (Meisch, 2000). They have sexual, asexual and mixed reproductive (bisexual and parthenogenesis) modes (Butlin et al., 1998). According to Ozawa (2013), sexual reproduction is a prevalent reproductive mode among ostracods while a part of species can capable of reproducing asexually (or so called parthenogenetic species). In addition, sexual dimorphism is also common on carapace and appendages of Podocopida (Ozawa, 2013).

However, Cohen and Morin (1990) revealed that sexual reproduction is common among marine ostracods while non-marine ostracods mostly reproduce asexually (Butlin et al., 1998). Indeed, the males of some species show restricted distribution and so they are known as geographical parthenogens (sexually and asexually reproducing populations of some species are available and the lineages of both sometimes coexist) (Butlin et al., 1998). Among the non-marine ostracods, for example, *Cavernocypris subterranean*, *Potamocypris villosa* and *Pseudocypridopsis clathrata* (Martens and Meisch, 1985; Karanovic, 1999; Külköylüoğlu and Vinyard, 2000) are known as geographical parthenogens. This can be explained by historical and ecological factors (Horne and Martens, 1999). Accordingly, Butlin et al. (1998) stated that *E. virens* and *H. incongruens* are the geographical parthenogens and their sexual populations are available in southern parts of their ranges. Further, Horne (1998) noticed that the 57 and 28% of species of Cypridoidea (286 spp) and Cytheroidea (50 spp), respectively, reproduce asexually in Europe. According to Meisch (2000), total of 159 species recorded from western and central Europe include 96 bisexual (with male), 46 parthenogenetic (male absent) and 17 geographical parthenogens. It is clearly seen that bisexual / parthenogenetic ratio is about larger than 2 but this ratio equals to ca. 1.2 in Turkey (Yılmaz, 2016).

The genus *Heterocypris* currently includes about 68 extant species (Martens et al., 2013). While 37 species have males, no males have been reported for 26 species and there is no clear information about whether males of the five species present (as shown in Table 1). Among the species of the genus, *H. incongruens*, which was also called as "horse trough" (Fryer, 1997) and it shows wide distribution in all zoogeographical regions (Karanovic, 2012; Martens et al., 2013). Considering its widespread distribution, its tolerance range is quite large for different environmental variables. Therefore, it is not only cosmopolitan (Meisch, 2000) but also is considered as cosmoecious species (Külköylüoğlu, 2013), indicating wide geographical distribution and wide tolerance ranges of the species. On the other hand, bisexual populations of this species have already been known from Palearctic and Nearctic regions including Algeria and Tunisia (Moniez, 1891), Romania (Masi, 1906), USA (Ohio) (Furtos, 1933), Spain (Margalef, 1953; Bellavere et al., 1999), southern France (Ohm and Remmert, 1955), Austria (near Vienna) (Löffler, 1961) and Portugal (Paulo and Moutinho, 1983). Besides, Meisch (2000) stated that the presence of bisexual population of *H. incongruens* with *H. barbara* in Czech Republic (Vávra, 1891), Hungary (Daday, 1900) and in eastern Germany (Klie, 1938) should be re-checked because the confusion with *H. barbara* cannot be clarified. In Turkey, male specimens have been reported in seven provinces (Gülen, 1985; Altınsaçlı, 1988; Külköylüoğlu et al., 1993; Gülen et al., 1994; Gülen et al., 1996; Özuluğ and Kılıç, 2002) (Figure 1). However, the presence of male in some of these studies is doubtful and so it should be check again. Thereby, the current study is aimed to i) discuss the presence of sexually reproducing population of *H. incongruens* in a man-made pond; ii) re-describe hemipenis of *H. incongruens* and iii) compare the hemipenis of the species with hemipenes of the other species in the genus whenever possible.

Figure 1. Distribution of bisexual populations of *H. incongruens* in Turkey (* previous reports; ** sampling site in the current study).

Table 1. Recent species of the genus *Heterocypris* and species with/without males. (*) represents species without any drawings of the hemipenis, and the question (?) means no information available about the presence of male.

	Species	with male	without male
1	*Heterocypris affinis* Klie, 1930	+	
2	*Heterocypris anitae* Battish, 1981	+	
3	*Heterocypris antillensis* Broodbakker, 1982	+	
4	**Heterocypris aurea* (Sars, 1895)	+	
5	*Heterocypris balnearia* (Moniez, 1893)	+	
6	*Heterocypris barbara* (Gauthier & Brehm, 1928)	+	
7	*Heterocypris bogotensis* Roessler, 1982		+
8	*Heterocypris bosniaca* Petkovski, Scharf & Keyser, 2000	+	
9	*Heterocypris calva* (Rome, 1965)		+
10	*Heterocypris capensis* (G.W. Müller, 1908)	+	
11	*Heterocypris carolinensis* (Ferguson, 1958)		+
12	*Heterocypris chandrai* (Arora, 1931)		+
13	*Heterocypris ciliata* (Thomson, 1879)		+
14	*Heterocypris congenera* (Vávra, 1897)	+	
15	*Heterocypris crenata* (Turner, 1893)	+	
16	*Heterocypris dubia* Sars, 1910		+
17	*Heterocypris euplocama* (Lowndes, 1931); (Martens, 1984)	?	?
18	*Heterocypris exigua* (Gauthier & Brehm, 1928)	+	
19	*Heterocypris favosa* Victor & Fernando, 1980		+
20	*Heterocypris fluviatilis* (Furtos, 1933)	+	
21	*Heterocypris gevgelica* Petkovski, Keyser & Scharf, 2000		+
22	*Heterocypris giesbrechti* (G.W. Müller, 1898)	+	
23	*Heterocypris glauca* (Furtos, 1933)	+	
24	*Heterocypris gregaria* (Skogsberg, 1917)	+	
25	**Heterocypris humilis* (Lindroth, 1953)	+	
26	*Heterocypris hyalina* Klie, 1930	+	
27	*Heterocypris imus* (Gauthier, 1934)	?	?
28	*Heterocypris incongruens* (Ramdohr, 1808)	+	
29	*Heterocypris indica* (Battish, 1981)		+
30	*Heterocypris kervillei* (Daday, 1911)		+
31	*Heterocypris leana* (Sars, 1896)		+
32	*Heterocypris luzonensis* Neale, 1981	+	
33	*Heterocypris makua* (Tressler, 1937)		+
34	*Heterocypris malini* Deb, 1983		+
35	*Heterocypris margaritae* Margalef, 1961	+	
36	*Heterocypris monodi* (Gauthier, 1938)	+	
37	*Heterocypris nicaraguensis* Hartmann, 1959	+	
38	*Heterocypris nuda* (Victor & Michael, 1975)		+
39	*Heterocypris nurrensis* Tagliasacchi-Masala	?	?
40	*Heterocypris nusbaumi* (Grochmalicki, 1911)		+
41	*Heterocypris oblonga* (Sars, 1924)		+
42	*Heterocypris ovularis* (Sars, 1924)	+	
43	**Heterocypris panningi* Brehm, 1934	+	
44	*Heterocypris parasimilis* Martens, 1994: (nom. nov. pro *H. similis* Klie, 1933 nec Wierzejski, 1893)	?	?
45	*Heterocypris persica* (Klie, 1937)	+	
46	*Heterocypris punctata* Keyser, 1976	+	
47	*Heterocypris putei* (Furtos, 1936)	+	
48	*Heterocypris reptans* (Kaufmann, 1900)	+	
49	*Heterocypris reticulatusa* (Tressler, 1937)		+
50	*Heterocypris rotundata* (Bronshtein, 1928)		+
51	*Heterocypris sabirae* Gülen, 1985	+	
52	*Heterocypris salaria* Hartmann, 1962		+
53	*Heterocypris salina* (Brady, 1868)	+	
54	*Heterocypris sanukiensis* Okubo, 2004	+	
55	*Heterocypris sarsi* (Brady, 1906)	+	
56	*Heterocypris setoensis* (Okubo, 1990)		+
57	*Heterocypris similis* (Wierzejski, 1893)	+	
58	*Heterocypris somalicus* (Masi, 1925)		+
59	*Heterocypris sydneia* (King, 1855)		+
60	*Heterocypris symmetricus* G.W. Müller, 1898	?	?

61	*Heterocypris symmetricus symmetricus* (G.W. Müller, 1898)	+		
62	*Heterocypris syriaca* (Daday, 1911)			+
63	*Heterocypris takedai* Okubo, 1973			+
64	*Heterocypris tatei* (Brady, 1886)	+		
65	*Heterocypris vandouwei* (Brehm, 1923)	+		
66	*Heterocypris vatia* De Deckker, 1981	+		
67	*Heterocypris wolffhuegeli* (Méhes, 1914)			+
68	*Heterocypris zugmayeri* (Brehm, 1914)			+

MATERIAL and METHODS

During an extensive field sampling (11 June and 8 September of 2010), total of 6 males and 59 females were collected with a plankton hand-net (150 μm) from Haydolar pond (a man-made pond: 41º23'42.3"N, 37º21'0.35"E, 821 m a.s.l.) in Pazarcık, Kahramanmaraş (Turkey) (Figure 2). The samples were fixed in 70% ethanol in a 250 ml of plastic bottle. This pond has been used as a water source for domestic animals for about last 50 years. There is no flowing water source into the pond from a spring or a creek. Therefore, it receives its water mainly from rainfall but during summer season villagers transport tape water into the pond to keep water level constant for domestic animals. On 11 June 2010, some physico-chemical variables of pond were measured as pH = 6.3 (Hanna HI-98150), electrical conductivity = 344.6 $\mu S.cm^{-1}$, salinity = 0.2 ‰, and water temperature = 25.6 ºC (YSI Model 85). In laboratory, individuals were hand sorted from sediments and then soft body parts of individual specimens were dissected in lactophenol solution, and the carapaces were kept in micropaleontological slides for Scanning Electron Microscope (SEM) photos (Figure 3) whenever possible. Drawing of soft body parts were prepared with a camera lucida (Olympus U-DA) attached to an Olympus BX51 microscope. We used terminology of Meisch (2000) to describe the chaetotaxy of limbs and taxonomic status of individuals. Also, previous drawing of the hemipenis of *H. incongruens* and other species of the genus were gathered from the literature (we directly used original drawings). The length of hemipenes and carapaces of species gathered from literature were measured by using the given scale. However, there was no any scale for carapaces and soft body parts in much of the former papers. Therefore, we are only able to provide measurement for hemipenes and carapaces of seven species and *H. incongruens*.

Figure 2. Haydolar pond (Kahramanmaraş, Turkey).

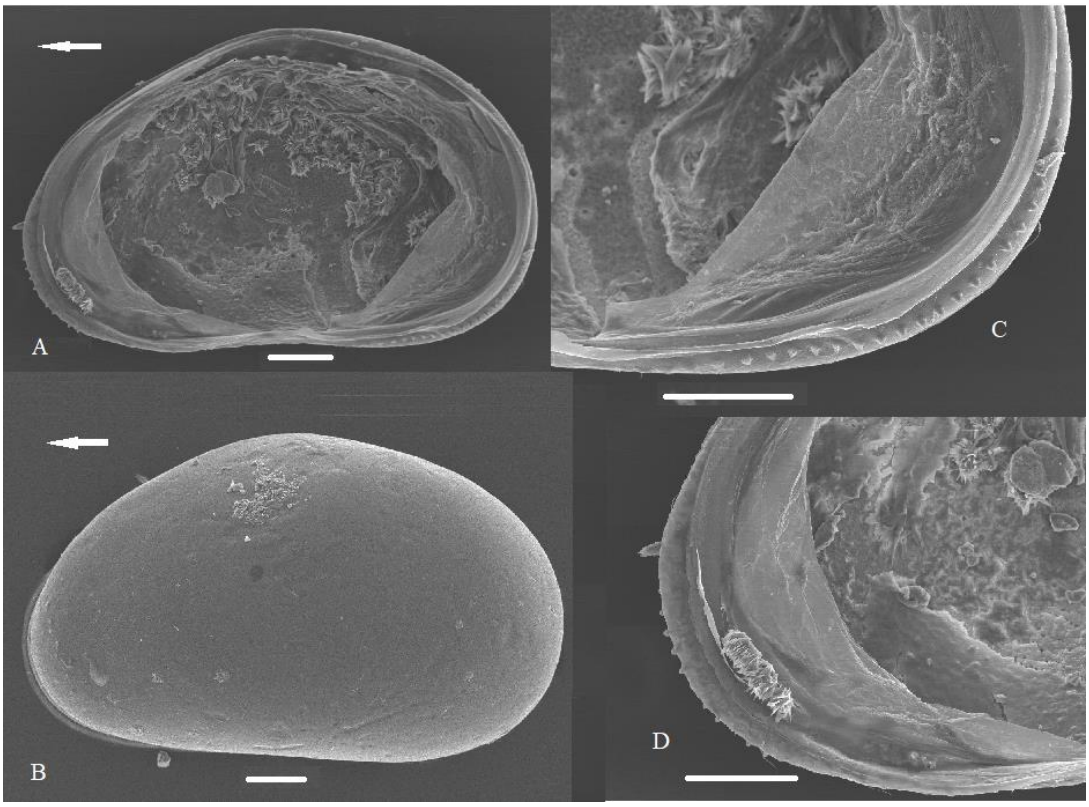

Figure 3. *Heterocypris incongruens* (male): A; right valve inside, B; left valve outside, C; right valve postero-ventral, D; right valve antero-ventral. Scale bar: 100 µm and arrows indicates anterior.

RESULTS

Total of 65 individuals (6 males and 59 females) of *H. incongruens* were collected with accompanying species of *Ilyocypris gibba*, *Potamocypris unicaudata* and *Trajancypris clavata*. Soft body parts of the male drawn herein are as shown in Figure 4. The earlier drawings of hemipenes of *H. incongruens* and other species of the genus obtained from the literature are shown in Figures 5 and 6, respectively.

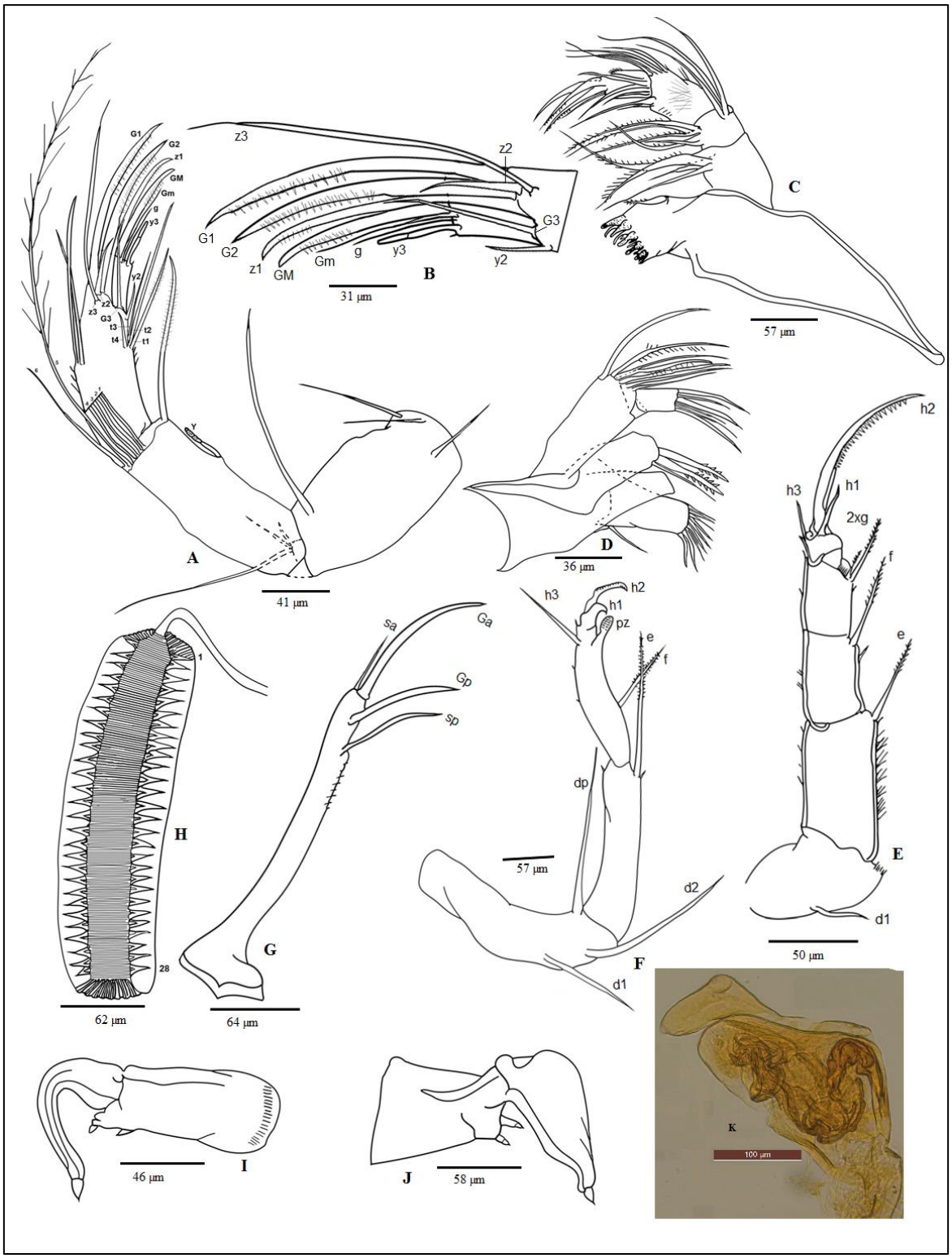

Figure 4. Soft body parts of male *H. incongruens* (the present study). A: A2; B: distal end of A2; C: mandibula; D: maxillula; E: walking leg; F: cleaning leg; G: uropod or furca; H: zenker's organ; I: right clasping organ; J: left clasping organ and K: hemipenis.

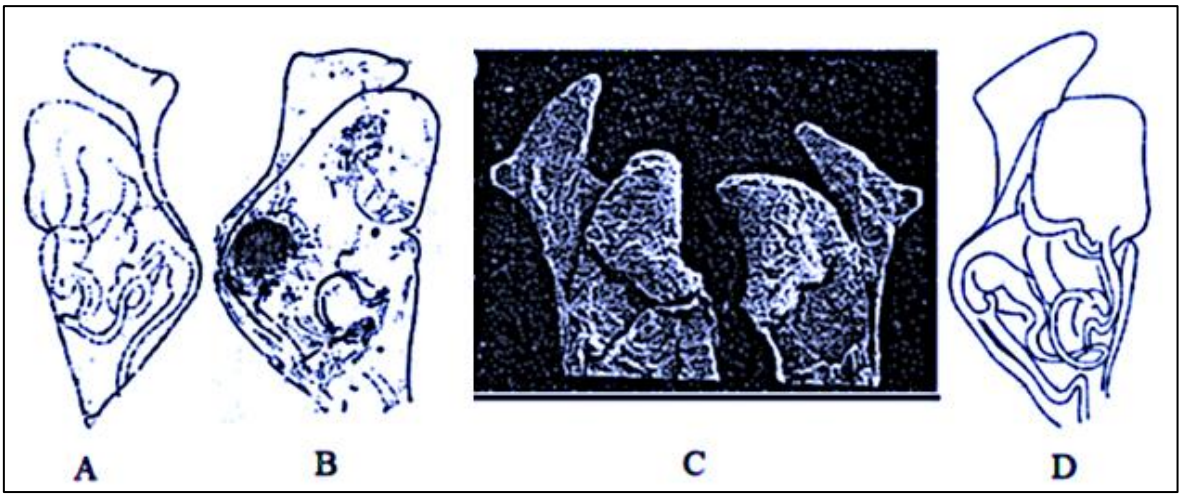

Figure 5. Drawings of the hemipenes of *H. incongruens* from previous works. A: from Daday (1900); B: from Gülen (1985); C: from Bellavere et al. (1999); D: from Meisch (2000).

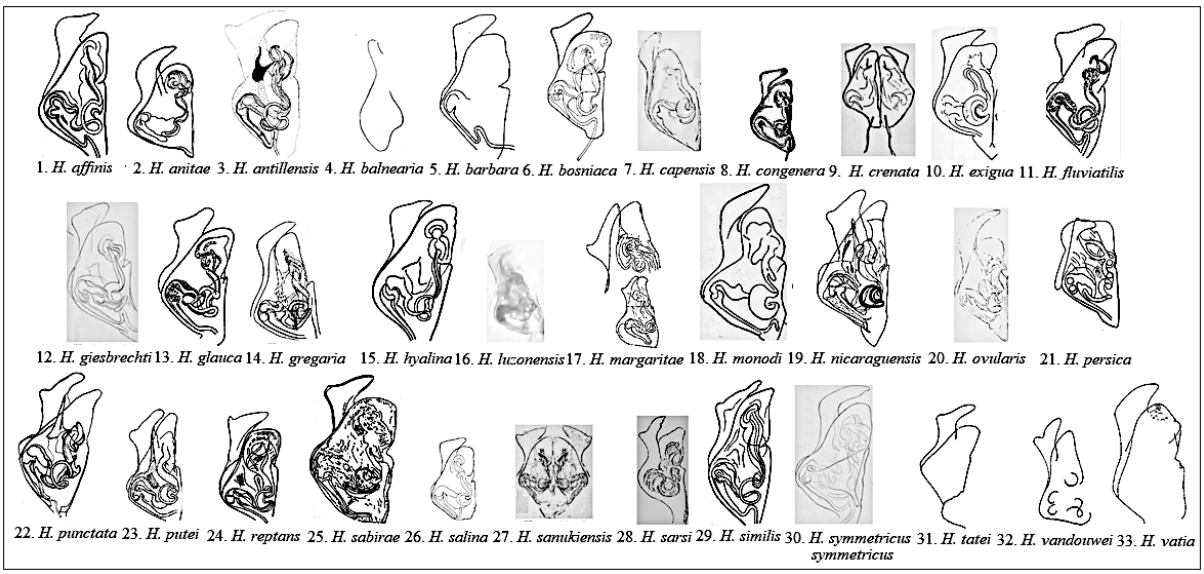

Figure 6. Hemipenes of other species in genus *Heterocypris*. Citation/s of 1 and 15 (Klie, 1930), 2 (Battish, 1981), 3 and 22 (Broodbakker, 1982), 4 (Moniez, 1893), 5 and 24 (Meisch, 2000), 6 and 26 (Petkowski et al., 2000), 7 (Sars, 1924a), 8 (Vàvra, 1895), 9 (Turner, 1893), 10 (Gauthier and Brehm, 1928), 11 and 13 (Furtos, 1933), 12 and 30 (Müller, 1898), 14 (Skosgberg, 1917), 16 (Neale, 1981), 17 (Margalef, 1961), 18 (Gauthier, 1938), 19 (Hartmann, 1959), 20 (Sars, 1924b), 21 and 24 (Klie, 1937), 23 (Furtos, 1936), 25 (Gülen, 1985), 27 (Okubo, 2004), 28 (Brady, 1906), 29 (Klie, 1933), 31 (De Deckker, 1979), 32 (Brehm, 1923) and 33 (De Decker, 1981).

Length of hemipenis (Figure 7) and carapaces for seven other species and for *H. incongruens* (from the current study and from Meisch (2000)) were tabulated in Table 2. The length of the hemipenis of *H. incongruens* is equal to 0.23 mm (N=2) when the length of the carapace equals to 1.21 mm in our samples. Thus, hemipenis / carapace ratio is about 0.19 mm when the range of this ratio is changed from 0.16 to 0.27 for other seven species (Table 2). Distal parts was not included when measuring the length of hemipenis because of i) this is a playful part and so length and position of it may change due to the erected or relaxed position of hemipenis and ii) damaging to this part during dissection of soft body parts of ostracods species for preparation of permanent slides.

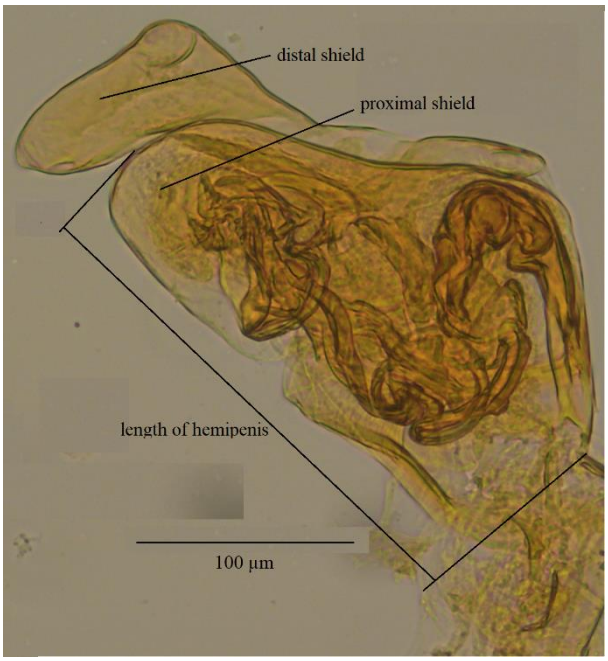

Figure 7. Hemipenis of *H. incongruens*.

Table 2. Length of hemipenis and carapaces (mm) and hemipenis/carapace ratio for seven species of the genus *Heterocypris* and of *H. incongruens* (* and ** represent individual herein and from Meisch (2000), respectively).

	Length of		Ratio
Species	**Hemipenis**	**Carapace**	**hemipenis/carapace**
H. anitae Battish, 1981	0.28	1.41	0.20
H. antillensis Broodbakker, 1982	0.27	1.20	0.23
H. barbara (Gauthier & Brehm, 1928)	0.29	1.20	0.24
H. exigua (Gauthier & Brehm, 1928)	0.15	0.79	0.19
H. punctata Keyser, 1976	0.30	1.11	0.27
H. tatei (Brady, 1886)	0.38	1.86	0.20
H. vatia De Deckker, 1981	0.28	1.70	0.16
H. incongruens (Ramdohr, 1808) **	0.34	1.25	0.27
H. incongruens (Ramdohr, 1808) *	0.23	1.21	0.19
Min	0.15	0.79	0.16
Max	0.38	1.86	0.27
Mean	0.28	1.30	0.22

DISCUSSION and CONCLUSION

In the present study, the presence of amphimictic (sexual) population of *H. incongruens* collected from a man-made pond was reported. However, this may not be limited within the ponds. Indeed, male individuals of the species were also encountered from hot water-spring and canal (Gülen, 1985). It shows that like parthenogenetic populations of the species, sexual populations may have a wide range of habitat preferences. Rossi et al. (2007) pinpointed about the morphological differences between the females of apomictic (asexual) and amphimictic populations of the same species (i.e., *H. barbara*) in the same locality. However, we did not observe significant morphological differences among the females of *H. incongruens*. Besides, the sex ratio (ca 1:10) herein was higher than the sex ratio (1:15) reported by Meisch (2000). This may demonstrate the sexually reproducing population of

H. incongruens in Haydolar pond. In other words, the male individuals in the current study are not occasional rare males.

Distal and proximal shields of the hemipenis (Figure 7) show variability (based on the 6 different drawings (one from the current study and 5 from the literature) and SEM photos) (Figure 5) among the individuals of male *H. incongruens* while no such variation is recorded for some of the species (among 35 species) of the genus in literature (e.g., *H. punctata* and *H. putei*). Indeed, the drawings of hemipenis of *H. incongruens* and *H. sabirae* in Gülen (1985) were not discernable to say that they belong to two different species. On the other hand, Okubo (1985) stated that male copulatory organ is a useful part to identify species because it does not show variability among individual of same marine species. However, this view may not be applied for many freshwater ostracods as seen among individual of *H. incongruens* (Figure 5) and in the individual of other species in genus *Heterocypris* (Figure 6). The distal shield of male *H. incongruens* described in here seems to be a human-foot shape with a prominent heel like structure (Figures 4K and 7) when this part showed variability for the same species in literature (Figure 5). Similarly, most of the poor drawings in the literature for the males of other 35 species show great variabilities in this part (Figure 6). Thereby, the information provided here may suggest that using hemipenis as a taxonomic character needs a great attention not only for the genus *Heterocypris* but also for the other genera of ostracods. This is because the shape of proximal and distal shields and the orientation of M-process may change due to the erection and comfortable situation of hemipenis (Klie, 1938). The closely resembling of all hemipenes of all the species of the genus *Heterocypris* seem to support that the genus is a monophyletic group.

The hemipenis / carapace ratio of *H. incongruens* herein (0.19 mm) is smaller than mean (0.22 mm) but it falls into the given range including other species (0.16 and 0.27) and ratio (0.27 mm) given in Meisch (2000) for the same species (Table 2). These suggest that hemipenis alone can reach up to one fifth of carapace length. Besides, the length of Zenker's organ herein (ca 0.28 mm) corresponds to the range (0.33-0.38 mm) provided by Matzke-Karasz et al. (2014) for *H. barbara*, *H. incongruens* and *H. rotundata* when their maximum sperm length ranged from 1.0 to 1.6 mm. This indicates Zenker's organ of *H. incongruens* is about 23% of carapace length. These findings moderately support the previous statements like that male reproductive system may reach up to a third of body volume (McGregor and Kesling, 1969) and at least 35 % of the body length (Cohen and Morin, 1990). McGregor and Kesling (1969) proposed that most of the entire body of ostracods are covered by the reproductive system. This reveals that reproductive system may have important role for taxonomic identification of this organisms. Besides, using some other parts of the hemipenis in taxonomic identifications, such as M-process (Meisch, 2000; Karanovic, 2012), accurately measured numeric values (as length of hemipenis, ratio of length of hemipenis to carapace) of the hemipenis may also be used as well. Therefore, when the taxonomic key is constructed, inclusion of such measurements of the hemipenis can help to make better identification.

Additionally, the soft parts of both sexes are of similarities but there can be small differences exist between them; therefore, sexual dimorphism is still obvious in males and females of *H. incongruens*. For example, aesthetasc Y on A2 is relatively mid-ventrally in male but lower mid-ventrally in female. G1 claws are similar in both sexes. However, G2 claw on the second antennae (A2) is smaller in females than males. Also, G3 claw reaching to the tips of G1 and Gm in female when it is about 1.5x and 2x of the length of terminal segment of A2 in male and female, respectively. In conclusion, we provide a re-description of the males of *H. incongruens* from Turkey, suggesting that the genus taxonomic status needs an urgent revision.

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