

Morphologic divergence and systematic relationship of Copepod in Turkish freshwater

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Received: 18. March 2014 / Accepted: 27. July 2014 / Available online: 04. October 2014 / Printed: June 2015

Abstract. The pattern of taxonomic relationship and morphological divergence of three copepod species *Thermocyclops crassus*, *T. dybowskii* and *T. vermifer* collected from three different localities in Mediterranean region of Turkey were investigated. A multivariate ANOVA using morphometric characters revealed highly significant intersample variation for most of the characters between species. In discriminant function analyses, the first discriminant function accounted for 65% and the second accounted for 35% of the between-group variability, showing clear between-species differentiation. Component loadings of discriminating variables showed that thorax segments of cephalothorax played an important role to discriminate *Thermocyclops* species. The Neighbour-joining analysis produced two main clustering, *T. vermifer* was closest taxa to *T. dybowskii* being the sister group to the *T. crassus* which was the most divergent in this group. In the correct assignment of individuals into their original sample, the proportion of correctly classified *T. crassus* to their original group was highest (100%), showing a clear separation from the other species.

Key words: morphologic divergence, systematic, copepod, *Thermocyclops*, freshwater.

Introduction

Genus *Thermocyclops* Kiefer, 1927 (Copepoda, Cyclopoida) is one of the most important among Cyclopidae that originated in the tropical region. The genus comprises more than fifty species and subspecies and widely distributed all around the world appearing the most diversified in Africa inhabiting surface fresh and brackish waters rarely occurring in ground waters (wells, anchialine caves) (Herbst 1986, Defaye et al. 1987, Reid 1989, Silva & Matsumura-Tundisi 2005). In many tropical water bodies, this genus represents the most important component of the zooplankton biomass. Most species of *Thermocyclops* genus are known for the tropical belt: south of African Sahara, tropical and subtropical regions of Asia, Americas and Australia. Furthermore, some species occur in temperate Eurasia. Only a very few species such as *T. crassus* (Fischer, 1853) are cosmopolitan while some others such as *T. oithonoides* (Sars, 1863) and *T. dybowskii* (Lande, 1890) are restricted to the Palaearctic region. *T. emini* (Mrázek, 1895), *T. consimilis* Kiefer, 1934, *T. neglectus* (Sars 1909), *T. macracanthus* (Kiefer, 1929), and *T. schmeili* (Poppe & Mrázek 1895) inhabit the African region (Dussart & Defaye, 1985). The trophic state of lakes or reservoirs seems to determine the occurrence of these species. In oligotrophic waters *T. minutus* (Lowndes, 1934) is more frequent found while, in eutrophic waters it is substituted by *T. decipiens* (Kiefer, 1929). In mesotrophic waters, the two species are found together, or alternately, according to the seasonal variation (Rocha et al. 1995). According to Hutchinson (1951), *T. decipiens* is a pioneering species of great dispersion and highly adaptable to colonize new environments, such as reservoirs and artificial water bodies (Baribwegure et al. 2000). There is still a conflict for the taxonomic description of *Thermocyclops vermifer* (Lindberg, 1935). This species is described with different names by different authors such as *Mesocyclops rylovi* var *vermifer* Lindberg, 1935, *Mesocyclops vermifer* Lindberg, 1938, 1939, *Mesocyclops (Thermocyclops) vermifer* Lindberg, 1942, *Thermocyclops rylovi vermifer* Lindberg, 1950, 1959, *Thermocyclops rylovi* cf. *vermifer* Löffler, 1961, *Thermocyclops vermifer* Tai & Chen, 1979. Average length of the species to the end of

caudal rami is 0.92 mm. This species was recorded from Sichuan, Yunnan, Hunan, Jiangxi, Zhejiang, Hubei, Shandong, Hebei and Tianjin of China, India, Afghanistan, Iran, Uzbekistan, Tadjikistan and southern Russia (the delta of the River Volga) (Tai & Chen 1979, Guo 1999).

Recently, Baribwegure and Dumont (1999) pointed out the possibility to use integumental pores as a tool for the cyclopoid copepods identification, as well as they provided the pore signature map in the genus *Thermocyclops*, using as a test case the species *T. emini* (Mrázek, 1895). Lately, Baribwegure et al. (2001), following the methodology of Baribwegure and Dumont (1999), combined the integumental pore signature with the description of other morphological characters to characterize *T. africana* Baribwegure, Thirion and Dumont, 2001, *T. neglectus* (Sars, 1909), and *T. oblongatus* (Sars, 1927).

Many aquatic organisms feed on copepods and they constitute second step of food chain in aquatic environment. Therefore, they are very important aquatic organisms but, there is little information available on descriptions and the taxonomic relationships of the copepod species.

Morphometric characters have been successfully used for taxonomic inferences. There are many well documented morphometric studies that provide evidence for taxonomic dilemmas (Lester & Pante 1992, Turan 2006). A new system of morphometric measurements called the truss network system (Strauss & Bookstein 1982) has been increasingly used for population and taxonomic studies (Lester & Pante 1992, Turan 2006). Therefore, in the present study, the pattern of taxonomic relationships and morphological divergence of one genus, including three species, *T. crassus*, *T. dybowskii*, and *T. vermifer*, living in the three different water resources in the Eastern Mediterranean region of Turkey, were investigated. Moreover this study is the first attempt of investigation taxonomic relationship of copepods by using multivariate analysis and the Truss network system.

Material and methods

Zooplankton samples were taken from three different localities in

Turkey. *T. vermifer* was collected from Aslantaş Dam Lake (37° 18' 31" N 36° 16' 03" E, 25 July 2001), *T. crassus* was collected from Yenişehir Lake (36° 14' 13" N 36° 34' 07" E, 13 July 2003) and *T. dybowskii* was collected from Berdan Stream (36° 55' 16" N 34° 55' 07" E, 20 March 2005) with horizontal and vertical draws by using 60 µm mesh size plankton net. Samples were replaced into glass jar and fixed with 4% formaldehyde.

Specimen were examined and photographed under an inverted microscope. The following taxonomic literature was used to identify the zooplankton specimen, Borutski (1963), Scourfield & Harding (1966), Dussart (1969), and Kiefer (1978). The samples are stored in the Plankton Laboratory, Faculty of Marine Sciences and Technology, Mustafa Kemal University.

The truss network system commonly used for fish body morphometrics (Strauss & Bookstein 1982) was used to construct a network on copepod body, twenty one landmarks determining 45 measurements were used on the copepod body, as illustrated in (Fig. 1). There were significant correlations between size and morphometric characters between the samples. Therefore the measurements were transformation to size-independent shape variables before the analyses. All measurements were standardized according to Eliot et al., (1995). In this formula, $M_{adj} = M (L_s/L_o)^b$ where M is the original morphometric measurement, M_{adj} the size adjusted measurement, L_o the Standard length of copepod and L_s the overall mean of Standard length for all copepod from all samples for each variable. The parameter b was used for each character from the observed data as the slope of the regression of $\log M$ on $\log L_o$, using all specimens. This allometric elimination was conducted with the MorFISH program (Turan & Oral 2005). Correlation coefficients between transformed variables and standard length were calculated to check if the allometric transformation was effective in removing the effect of size in the data.

Discriminant Function Analysis (DFA) was used for multivariate analysis of three copepod species. Discriminant function analysis combines a selection of body measures in a linear fashion to produce a mathematical function which can be used to classify individuals into groups. Discriminant function scores were used in hierarchical cluster analyses using SPSSv13.0. In hierarchical cluster analyses UPGM dendrogram was used that does not plot actual distances but rescales the distance to numbers between 0 and 25. The degree of similarity among samples in the overall analysis and relative importance of each measurement for group separation were assessed by stepwise DFA with jack-knifed classification. Population centroids with 95% confidence ellipses derived from the DFA were used to visualize relationships among the individuals of groups.

Results

Analysis of variance test revealed highly significant inter sample variation from the characters, 10_11, 1_3, 2_4, 18_20. In discriminant function analysis 65% of total variation was presented in the first DF, and remaining 35% of total variation presented in the second discriminant function. Plotting the first and second discriminant functions highly separated the tree species from each other on the discriminate space (Fig. 2). In the neighbour joining tree *T. dybowskii* and *T. vermifer* clustered together and was sister group to *T. crassus*. Component loadings of discriminating variables showed that 5_6, 10_11, 2_4 and 18_19 have high contribution to discriminate *Thermocyclops* species and play an important role to distinguish these species (Table 1). The amount of morphological divergence was highest between *T. crassus* and *T. vermifer*, and lowest between *T. dybowskii* and *T. vermifer* (Table 2).

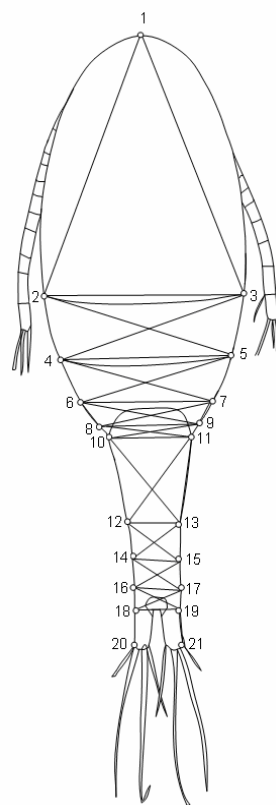


Figure 1. Application of the truss network system on the body of copepod

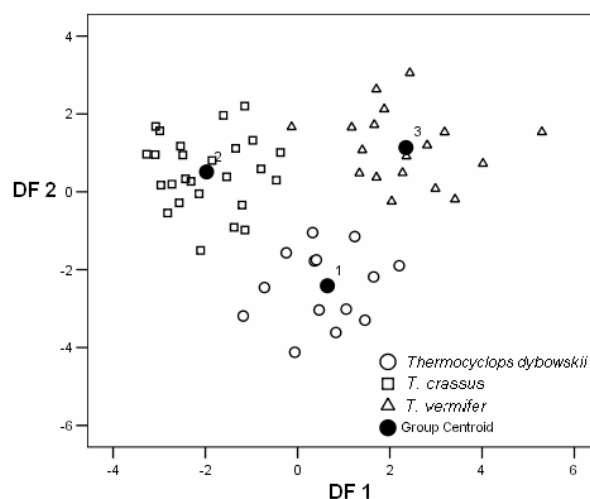


Figure 2. Plotting DF scores for morphometric analysis.

The Neighbour-joining analysis of morphological data produced two main clustering (Fig. 3). *T. vermifer* was closest taxa to *T. dybowskii* being the sister group to the *T. crassus* which was the most divergent in this group.

The overall assignment of individuals into their original sample by the DFA was 92.9% (Table 3). The proportion of correctly classified *T. crassus* to their original group was highest (100%), showing a clear separation from all others. Whereas, *T. dybowskii* and *T. vermifer* showed more similarity than *T. crassus*.

Table 1. Component loadings of discriminating variables. Variables ordered by absolute size of correlation within function.
*Largest absolute correlation between each variable and any discriminant function.

Characters	Function	
	1	2
5_6	-0,082*	0,076
10_11	0,126	-0,299*
2_4	0,088	-0,217*
18_19	0,023	-0,211*
18_20	0,026	-0,211*
10_13	0,057	-0,210*
20_21	0,043	-0,205*
11_13	0,082	-0,205*
19_21	0,010	-0,201*
19_20	-0,004	-0,191*
2_5	0,095	-0,185*
9_10	0,062	-0,180*
4_6	-0,087	-0,177*
2_3	0,046	-0,176*
9_11	0,072	-0,173*
17_18	0,014	-0,171*
14_15	0,031	-0,171*
8_11	0,026	-0,168*
21_1	0,079	-0,167*
1_21	0,079	-0,167*
13_15	0,054	-0,167*
8_9	0,041	-0,166*
4_5	-0,001	-0,165*
7_8	0,019	-0,164*
13_14	0,051	-0,163*
12_15	0,069	-0,162*
12_14	0,068	-0,161*
12_13	0,039	-0,160*
16_19	0,002	-0,160*
8_10	0,083	-0,158*
5_7	0,042	-0,157*
1_3	0,152	-0,155*
3_4	-0,024	-0,152*
15_16	0,009	-0,151*
14_16	0,018	-0,148*
16_18	0,021	-0,147*
4_7	0,011	-0,142*
1_2	0,094	-0,138*
3_5	-0,008	-0,138*
6_7	0,045	-0,137*
7_9	0,026	-0,135*
6_8	0,053	-0,133*
16_17	0,033	-0,131*
14_17	0,028	-0,099*
6_9	0,057	-0,099*
15_17	0,009	-0,091*
11_12	0,044	0,050*

Table 2. Euclidean morphological distance between species.

Species	<i>Thermocyclops dybowskii</i>	<i>Thermocyclops crassus</i>	<i>Thermocyclops vermifer</i>
<i>Thermocyclops dybowskii</i>	-		
<i>Thermocyclops crassus</i>	18.52	-	
<i>Thermocyclops vermifer</i>	12.24	21.79	-

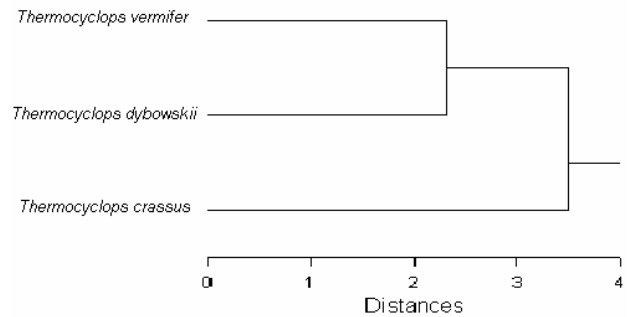


Figure 3. Neighbour-joining analysis of morphological data.

Table 3. Correct classification of individuals in to their original species.

Species	Predicted Group Membership			
	1	2	3	Total
<i>Thermocyclops dybowskii</i> (1)	93	0	7	100
<i>Thermocyclops crassus</i> (2)	0	100	0	100
<i>Thermocyclops vermifer</i> (3)	6	0	94	100

Discussion

In the present investigation, the taxonomic status of *T. crassus*, *T. dybowskii* and *T. vermifer* within the genera *Thermocyclops* were preliminary investigated. The amount of morphologic divergence was lower between *T. dybowskii* and *T. vermifer*, and higher between *T. crassus* and *T. vermifer* within this genus. Therefore, *T. vermifer* showed least morphological differentiations and showed close morphological similarity to *T. dybowskii*. These results lead us to reinvestigate the taxonomic status of the tree species. This can be achieved with detailed investigation using both morphological and molecular genetic markers. Component loadings of discriminating variables showed that 5_6, 10_11, 2_4 and 18_19 have high contribution to discriminate *Thermocyclops* species and play an important role to distinguish these species. Hence these characters play an important role for the identification of these species. Taxonomic description of a species has commonly relied on description of unique sets of morphological characters. Morphometric measurements from thorax of copepod (5_6, 2_4) and abdomen segments (10_11, 18_19) were the most discriminative characters in the present classification. Such characters are phylogenetically informative to distinguish the species of the *Thermocyclops*.

Molecular genetic markers such as microsatellite and mtDNA should be used (Graves 1998, Turan et al. 1998, Shaw et al. 1999) to reveal the genetic component of phenotypic discreteness between species.

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