ZOOCENOSES AS A COMPONENT OF BIOGEOCENOSIS

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IDENTIFICATION OF SIBLING SPECIES IN THE GENUS *EUCYCLOPS* FROM WATER-BODIES OF UKRAINE AND RUSSIA USING CROSSBREEDING STUDIES

Crossbreeding experiments were used to estimate cryptic species in water bodies of Ukraine and Russia because the most useful criterion in species independence is reproductive isolation. The problem of cryptic species in the genus *Eucyclops* was examined using interpopulation crosses of populations collected from Baltic Sea basin (pond of Strelka river basin) and Black Sea basin (water-reservoires of Dnieper, Dniester and Danube rivers basins).

The results of reciprocal crosses in *Eucyclops serrulatus*-group are shown that *E. serrulatus* from different populations but from water bodies belonging to the same river basin crossed each others successfully. The interpopulation crosses of *E. serrulatus* populations collected from different river basins (Dnipro, Danube and Dniester river basins) were sterile. In this group of experiments we assigned evidence of sterility to four categories: 1) incomplete copulation or absence of copulation; 2) nonviable eggs; 3) absence of egg membranes or egg sacs 4) empty egg membranes. These crossbreeding studies suggest the presence of cryptic species in the *E. serrulatus* inhabiting ecologically different populations in many parts of its range.

The same crossbreeding experiments were carries out between *Eucyclops serrulatus* and morphological similar species – *Eucyclops macruroides* from Baltic and Black Sea basins. The reciprocal crossings between these two species were sterile. Thus taxonomic heterogeneity among species of genus *Eucyclops* lower in *E. macruroides* than in *E. serrulatus.*

The interpopulation crosses of *E. macruroides* populations collected from distant part of range were fertile. These crossbreeding studies suggest that *E. macruroides* species complex was evaluated as more stable than *E. serrulatus* species complex.

Key words: *crossbreeding, reproductive isolation, Eucyclops, cryptic species.*

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ОБНАРУЖЕНИЕ ВИДОВ-ДВОЙНИКОВ В РОДУ *EUCYCLOPS* ИЗ ВОДОЕМОВ УКРАИНЫ И РОССИИ ПУТЕМ ГИБРИДОЛОГИЧЕСКОГО АНАЛИЗА

Для выявления видов-двойников из водоемов Украины и России использовался метод реципрокных скрещиваний. Реципрокные скрещивания между географически удаленными популяциями *Eucyclops serrulatus* из водоемов, которые принадлежат к одному речному бассейну, были успешными, в то время как при скрещивании популяций из водоемов бассейна Днепра с популяциями из бассейна Днестра и Дуная были выявлены свидетельства стерильности отношений у этих видов. Этот факт, возможно, указывает на наличие криптических видов в группе *Eucyclops serrulatus* в этом регионе. Скрещивания *E. serrulatus* и *E. macruroides* оказались стерильными. Скрещивания между одноименными популяциями *E. macruroides*, но происходящими из отдаленных участков ареала, были успешными. В результате *E. macruroides* оценивается как более стабильный комплекс, чем *E. serrulatus* комплекс.

Ключевые слова: скрещивание, репродуктивная изоляция, Eucyclops, криптические виды.

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ВИЯВЛЕННЯ ВИДІВ-ДВІЙНИКІВ В РОДІ *EUCYCLOPS* ІЗ ВОДОЙМ УКРАЇНИ ТА РОСІЇ ШЛЯХОМ ГІБРИДОЛОГІЧНОГО АНАЛІЗУ

Для виявлення морфологічно схожих видів (криптичних, видів-двійників) із водойм України та Росії використовували метод реципрокних схрещувань, оскільки найбільш важливим критерієм для оцінки видової самостійності є репродуктивна ізоляція. Для виявлення криптичних видів роду *Eucyclops* проводили реципрокні схрещування між географічно віддаленими популяціями із басейнів Балтійського (ставок в басейні річки Стрілка) та Чорного морів (водойми в басейнах річок Дніпра, Дністра та Дунаю).

Результати реципрокних схрещувань в групі *Eucyclops serrulatus* вказують, що особини цього виду із різних популяцій, але із водойм, що належать до одного річкового басейну схрещувалися успішно. Схрещування між особинами *E. serrulatus* із водойм, що належать до різних річкових басейнів були стерильними. В цій групі схрещувань спостерігались наступні докази стерильності: 1) відсутність копуляції, 2) розвиток слабко розвинених яйцевих мішків із нежиттєздатними яйцями, 3) виділення гомогенної яйцевої маси, 4) формування пустих

яйцевих мембран. Виявлені риси стерильності відносин експериментальних пар при схрещуванні особин із різних річкових басейнів України дають підстави передбачати наявність криптичних видів в межах таксону *E. serrulatus*.

Аналогічні досліди по схрещуванню були проведені для пари морфологічно близьких видів роду *Eucyclops – E. serrulatus* та *E. macruroides*. Реципрокні схрещування між цими видами виявилися стерильними. Таким чином, серед видів роду *Eucyclops*, що мешкають у водоймах України та Росії, таксономічна неоднорідність *E. macruroides* значно нижча, ніж *Eucyclops serrulatus*.

Результати реципрокних схрещувань в групі *E. macruroides* вказують, схрещування між однойменними популяціями цього виду, що походять із віддалених частин ареалу, були фертильними. Ці результати вказують на те, що комплекс *E. macruroides* більш стійкий ніж комплекс *E. serrulatus*.

Ключові слова: *схрещування, репродуктивна ізоляція, Eucyclops, криптичні види.*

INTRODUCTION

Eucyclops serrulatus Fischer, 1853 is a widespread species of cyclopids. The detailed description of the species with criterions for distinguishing from similar in morphology species is presented in publications (Monchenko, 1974; Alekseev, 1995; Alekseev *et al.*, 2006; Alekseev & Defaye, 2011).

There is supposition, that cosmopolitan species to which *E. serrulatus* belongs in different part of range are represented by sibling species (Alekseev & Defaye, 2011). This fact follows also from established before distinction in susceptibility of *Eucyclops* s. lat. local population to helminthes invasions (Monchenko, 2003).

The presence of cryptic species among the cyclopids was revealed in species groups *Acanthocyclops vernalis, A. americanus*, *Diacyclops bicuspidatus* (Kochina, 1987; Monchenko, 2000; Monchenko, 2003). The studies on presence of cryptic species among *Eucyclops* are incomplete (Monchenko, 2003; Alekseev & Defaye, 2011). Inasmuch as the biological species concept is not realized the issue of monotypic species *Eucyclops serrulatus* remains open.

In the present study, we use interbreeding studies to look for cryptic species in *Eucyclops serrulatus* s.lat. The crossbreeding experiments utilized seven populations of *E. serrulatus* belonging to the same water basin and different river basins (the Dnipro, Dnister and Danube river basins). Additionally the question of intraspecies heterogeneity of other species of genus *Eucyclops – E. macruroides* from water-bodies belonging to Basins of the Baltic and Black Seas was studied.

MATERIAL AND METHODS

According to contemporary notions the most useful criterion in species independence is reproductive isolation. The problem of cryptic species in the genus *Eucyclops* was examined using interpopulation crosses of population collected from Baltic Sea basin (pond of Strelka river basin) and Black Sea basin (water-reservoires of Dnieper, Dniester and Danube rivers basins). The sites from which specimens of the genus *Eucyclops* were obtained are shown in Table 1.

The samples containing living specimens of cyclopids were taken at a depth 0,1–1 m into plastic vessels (0,5–l) and brought to the laboratory without preliminary fixation. This samples examined under dissecting microscope (MBS-9). The living specimens of *Eucyclops* identified under light microscope (Olympus BX51 and MBI-11). The females of *Eucyclops* with egg sacs were isolated for monocultures. The males of *Eucyclops* and IV–V stage copepodids were isolated and reared for following reciprocal crosses. The samples containing living specimens of cyclopids from different sites were used for mass cultures. All parental population produced normal offspring during the 5–6 months following the experiments. Adult males and copepodid IV–V stage females were removed from the monocultures and mass cultures for following reciprocal crosses.

The mass cultures reared in beakers (200–300 ml containing living specimens of cyclopids) filled by aged tap water. For rearing monocultures we used small dishes (10–15 ml). The males and IV–V stage copepodids were reared on object-plates (without covered glass).

Table 1

The sites from which specimens of the genus *Eucyclops* were obtained

№	River basin	Sites
The Black Sea basin		
1	The Dnipro river basin	Dnipro River (Kyiv, Ukraine), Psel River (Sumy, Ukraine), Sluch River (the Khmelnytsky region, Ukraine), Sula River (the Sumy region, Ukraine), pond in Pushcha-Voditsa (Kyiv, Ukraine), pond near village Khotov (vicinity of Kyiv, Ukraine)
2	The Dniester river basin	Dniester River (Odessa region, Ukraine); ponds in national preserve ”Roztocze”(Lviv region, Ukraine)
3	The Danube river basin	Pond (Transcarpathian region, Ukraine)
The Baltic Sea Basin		
1	The Strelka river basin	Pond (village Ropsha, Leningrad region, Russia)

The method of individual cultivation of cyclopids was developed for identification of sibling species in *Diacyclops bicuspidatus* (Monchenko, 2000). It base on rearing separate speciments on IV-V copepod stages in drop of water on object-plates which is situated in moist chamber (Petri dish with moist cotton).

For crossbreeding experiments to virgin females after confirming species identification added male from other population.

Copepods were fed from the infusoria laboratory culture three times a week composed of *Paramecium* sp. and wild caught heterotrophic flagellates. Simultaneously, half of the water volume of the vessel was replaced with aged tap water.

All interbreeding crosses were performed at room temperature (15–20 ºC). We observed animals daily for maturity and then performed the cross immediately. The experimental designs of these crossbreedings are shown in Table 1–3. When referring to crosses, the female of the pair is always listed first. The lack of synchronous development among the different population and unequal sex ratio resulted in small numbers of replicates for pairings. A total 32 mature females were paired in the interpopulation crosses.

We assigned evidence of sterility to four categories: 1. Females produced one or two weakly developed egg sacs which contained 1–5 eggs per sac. This reduced clutch size contrasts with the typical size of 50–100 eggs per clutch in the parental (control) lines. Eggs were nonviable and after 10–12 days they were covered by fungi. It is considered the most common evidence of sterility; 2. Empty egg membranes; 3. Absence of egg membranes or egg sacs; 4. Incomplete copulation or absence of copulation.

RESULTS

The results of the crosses are shown in Table 2–4. Cultures of all individual parental populations lived and reproduced for 5–6 months following the crosses which resulted in the F_1 and F_2 hybrids.

The results of reciprocal crosses between geographic remote populations of *E. serrulatus* are shown in Table 2. Four interpopulation crosses between populations of *E. serrulatus* from the same river basin demonstrated reproductive fertility: 1. pond in Pushcha-Voditsa × Psel River, and its reciprocal cross, 2. Sluch River and Sula River, and its reciprocal cross, 3. Sluch River × pond in Pushcha-Voditsa and its reciprocal cross, 4. pond in national preserve "Roztocze" × Dnister River and its reciprocal cross. In these cases the females produced 3–6 pair of egg sacs. The time of appearance of egg sacs on female was 1–7 days. The eggs were viable and hatched into nauplii. Embryonal development of F_1 hybrids lasted 2–3 days.

Seven interpopulation crosses between populations of *E. serrulatus* from different river basins demonstrated sterility (Table 2): 1. Sula River × Dnister River, and its reciprocal cross, 2. Psel River × pond in national preserve "Roztocze", and its reciprocal cross, 3. pond in Pushcha-Voditsa × Dnister River and its reciprocal cross, 4. Sluch River × pond in national preserve "Roztocze", and its reciprocal cross, 5. Dnister River × Psel River and its reciprocal cross, 6. pond in national preserve "Roztocze" × Sula River and its reciprocal cross, 7. pond (the Danube river basin) × Dnipro river and its reciprocal cross. In these cases adult males and females kept together 10–30 days but more often there are no couplings. Copulations were observed in 23 % pairs from 13 investigated pairs. In these cases females produced: 1) one or two weakly developed egg sacs which contained 1–4 eggs per sacs. Eggs were nonviable and did not hatch into nauplii; or 2) homogenous egg mass or 3) empty egg membranes.

Table 2

Experimental design and data on fertility of crossbreeding pairs from populations of *Eucyclops serrulatus* from rivers belonging to Dnipro, Dniester and Danube river basins

Female / Male		The Dnipro river basin				The Dniester river basin	
		Psel River	Sula River	pond in Pushcha-Voditsa	Dnipro River	Dniester River	ponds in national preserve "Roztocze"
The Dnipro river basin	Psel River						Sterile 1 pairs
	Sula River					Sterile 2 pairs	
	pond in Pushcha-Voditsa	fertile 1 pair				Sterile Empty egg membranes 1 pairs	
	Sluch River		fertile 1 pair	fertile 2 pairs			Sterile 1 pairs
The Dniester river basin	Dniester River	Sterile homogenous egg mass 3 pairs	Sterile 1 pairs	Sterile nonviable eggs 2 pairs			
	ponds in national preserve "Roztocze"		Sterile nonviable eggs 1 pairs			fertile 2 pairs	
The Danube river basin	pond				Sterile 1 pairs		

The same crossbreeding experiments were carries out between allied species *E. serrulatus* and *E. macruroides* from Baltic and Black Sea basins. The results of the crosses are shown in Table 3. Five crosses demonstrated sterility: 1. Psel River × Sula River and its reciprocal cross, 2. Dnipro River × Sula River and its reciprocal cross, 3. Sula River × pond in national preserve "Roztocze" and its reciprocal cross, 4. pond in national preserve "Roztocze" × pond (the Strelka river basin) and its reciprocal cross, 5. Psel River × pond (the Strelka river basin) and its reciprocal cross. Males of *Eucyclops macruroides* in general do not seem to purposefully choose their mate for copulation and sometimes attempt to copulate with females of *Eucyclops serrulatus*. But the crosses were sterile. Thus the reciprocal crossings between *Eucyclops serrulatus* and morphological similar species – *Eucyclops macruroides* demonstrated sterility that was recorded in previous publications (Lowndes, 1932).

Table 3

Experimental design and results of the crosses of crossbreeding pairs from populations *E. serrulatus* and *E. macruroides* from Baltic and Black Sea basins

<table>
<tr><td colspan="3" rowspan="2">Female
Male</td><td colspan="2">*E. serrulatus*</td><td colspan="2">*E. macruroides*</td></tr>
<tr><td>The Dnipro river basin</td><td>The Dniester river basin</td><td>The Dnipro river basin</td><td>The Strelka river basin</td></tr>
<tr><td colspan="3">Male</td><td>Psel River</td><td>ponds in national preserve ”Roztocze”</td><td>Sula River</td><td>pond</td></tr>
<tr><td rowspan="3">*E. serrulatus*</td><td rowspan="2">The Dnipro river basin</td><td>Psel River</td><td></td><td></td><td>Sterile no copulation 1 pairs</td><td>Sterile no copulation 1 pairs</td></tr>
<tr><td>Dnipro River</td><td></td><td></td><td>Sterile no copulation 1 pairs</td><td></td></tr>
<tr><td>The Dniester river basin</td><td>ponds in national preserve ”Roztocze”</td><td></td><td></td><td></td><td>Sterile no copulation 1 pairs</td></tr>
<tr><td>*E. macruroides*</td><td>The Dnipro river basin</td><td>Sula River</td><td>Sterile 2 pairs</td><td>Sterile no copulation 1 pairs</td><td></td><td></td></tr>
</table>

The problem of cryptic species in *Eucyclops macruroides* was examined using interpopulation crosses of three populations collected from a: 1) Sula River (the Dnipro river basin), 2) pond (the Strelka river basin), pond (the Danube river basin). The results of reciprocal crosses between geographic remote populations of *E. macruroides* are shown in Table 4.

Table 4

Experimental design and data on fertility of crossbreeding pairs from populations of *Eucyclops serrulatus* from rivers belonging to Dnipro, Dniester and Danube river basins

Female / Male	The Dnipro river basin, Sula River	The Danube river basin, pond
The Strelka river basin, pond	fertile 5 pairs	
F_1 (♂ The Strelka river basin x ♀ Sula River)		fertile 1 pairs

All interpopulation crosses demonstrated reproductive fertility. Five pairings of the Sula River (the Dnipro river basin) × pond (the Strelka river basin) produced viable eggs which hatched into nauplii. Copulations were observed during 1–60 min after male was added to female. After 1–3 days females produced well-developed egg sacs. The females produced 2–5 pair of egg sacs. The time of appearance of new egg sacs on female was 2–9 days. Embryonal development of F_1 hybrids lasted 2–3 days. To obtain F_1 hybrids (the nauplii of pond (the Strelka river basin) × Sula River cross), were reared to adult and used for following experiments. Pairings of one F_1 hybrids (the nauplii of pond (the Strelka river basin) × Sula River cross) × pond (the Danube river basin) produced viable eggs which hatched into nauplii.

CONCLUSION

1. The results of reciprocal crosses in *Eucyclops serrulatus*-group are shown that *E. serrulatus* from different populations but from water bodies belonging to the same river basin crossed each others successfully. The interpopulation crosses of *E. serrulatus* populations collected from different river basins (Dnipro, Danube and Dniester river basins) were sterile, as evidenced by either nonviable eggs, empty egg membranes or incomplete copulations. These crossbreeding studies suggest the presence of cryptic species in the *E. serrulatus* inhabiting ecologically different populations in many parts of its range.

2. The reciprocal crosses between *E. serrulatus* and morphological similar species *E. macruroides* were sterile. Thus taxonomic heterogeneity among species of genus *Eucyclops* lower in *E. macruroides* than in *E. serrulatus.*

3. The interpopulation crosses of *E. macruroides* populations collected from distant part of range were fertile. These crossbreeding studies suggest that *E. macruroides* species complex was evaluated as more stable than *E. serrulatus* species complex.

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