

**KARYOTYPE, MORPHOLOGY, AND REPRODUCTION ABILITY
OF THE PRUSSIAN CARP, *CARASSIUS GIBELIO* (ACTINOPTERYGII: CYPRINIFORMES:
CYPRINIDAE), FROM UNISEXUAL AND BISEXUAL POPULATIONS IN POLAND**

Alicja BOROŃ^{1,2}, Jolanta SZLACHCIAK¹, Dorota JUCHNO^{1*}, Anna GRABOWSKA¹,
Bartosz JAGUSZTYN³ and Katarzyna PORYCKA¹

¹ *Division of Zoology, University of Warmia and Mazury, Olsztyn, Poland*

² *Polish Academy of Science, Department of Mammalian Research, Białowieża, Poland*

³ *Department of Biotechnology, Faculty of Biotechnology, University of Rzeszów, Kolbuszowa, Poland*

Boroń A., Szlachciak J., Juchno D., Grabowska A., Jagusztyn B., Porycka K. 2011. Karyotype, morphology, and reproduction ability of the Prussian carp, *Carassius gibelio* (Actinopterygii: Cypriniformes: Cyprinidae), from unisexual and bisexual populations in Poland. *Acta Ichthyol. Piscat.* 41 (1): 19–28.

Background. The majority of the European populations of the Prussian carp, *Carassius gibelio*, consist predominantly of females reproducing by gynogenesis but include a small portion of males. Mechanisms for the occurrence of males in natural populations and their functions are still unclear. The aims of this study were to show the ploidy level by karyotype, some taxonomic characteristics by analysis of morphology, and the reproductive potential by histology of gonads of Prussian carp individuals of a certain age from two types of naturally existing unisexual triploid and a diploid-polyploid populations.

Materials and methods. In total 31 specimens from three different populations inhabiting the Vistula River basin, the Baltic Sea watershed, have been analyzed. Kidneys were used to prepare chromosome slides using standard procedures. Air-dried slides were stained with Giemsa solution. For each fish, 23 metric characteristics of the body were measured. Selected meristic characteristics (external and internal) were determined. The gonads were fixed in buffered formalin or in Bouin's solution; histological sections were stained with haematoxylin and eosin (HE). The developmental stages of germ cells and gonads were determined according to Rinchar and Kestemont and Billard.

Results. All individuals of two populations were triploid females with high chromosomal variability from 150 to 160 chromosomes (and microchromosomes). Among individuals of the third population, males and females of $2n = 100$ and one triploid male $3n = 160$ (151–162) chromosomes were detected. The individuals from analyzed populations differed in the number of: branched rays in the dorsal fin, lateral line scales, serration in dorsal *D* and anal *A* fin rays, and vertebrae. Gonad histology of the ovaries indicated the females were mature at the age of 3. Testes morphology of diploids and one triploid male indicated their reproductive abilities.

Conclusion. The results of the study confirmed the tendency to increase the number of males in *C. gibelio* populations. In several or some populations apart from diploid males a small number of fertile triploid males occur. High chromosomal variability within the females from unisexual populations may be influenced by their different mode of origin. The future research should be focused on karyological identification of other *C. gibelio* populations to explain the origin of triploid males and their role in reproduction.

Keywords: *Carassius gibelio*, Prussian carp, gonad histology, karyotype, males, morphology, polyploidy

INTRODUCTION

The Prussian carp, *Carassius gibelio* (Bloch, 1782), is widely distributed in Europe, Siberia, and Asia. In the eastern part of the region, it is represented mainly by bisexual populations, with some local unisexual polyploid populations, which frequently coexist with bisexual ones (Fan and Liu 1990, Fan and Shen 1990). The majority of the European populations of this species have been considered

unisexual, consisting of triploid females reproducing by gynogenesis (Sofradžija et al. 1978, Penaz et al. 1979, Fister and Soldatovic 1989, Tóth et al. 2005). The coexistence of triploid females and diploid females and males of *C. gibelio* was reported for the first time by Cherfas (1966). Several recent papers have found that some other natural unisexual populations of this species consist of a minor but significant portion of males (Boroń 1994, Boroń and Boroń 1996,

* Correspondence: Dr Dorota Juchno, Katedra Zoologii, Uniwersytet Warmińsko-Mazurski w Olsztynie, ul. Oczapowskiego 5, 10-718 Olsztyn, Poland, phone: (+48) 89 523 3319; fax: (+48) 89 523 3261, e-mail: juchno@uwm.edu.pl.

Zhou et al. 2000, Devlin and Nagahama 2002, Tóth et al. 2005). Mechanisms for the occurrence of a small and variable proportion of males in natural populations are still unclear. All the eggs of the triploid Prussian carp developed gynogenetically into females when stimulated by sperm from other species. A small proportion of eggs developed into males when 'fertilized' with sperm from the male of the same species (Shen et al. 1983). Heterologous sperm of other fish species apart from activating eggs into embryological development was able to contribute to the phenotype of the gynogenetic offspring of triploid females of *C. gibelio* (see Yi et al. 2003). This type of gynogenesis has been referred to as allogynogenesis.

Diploid individuals of Prussian carp were reported to have varying numbers of chromosomes, ranging from $2n = 94$ to $2n = 100$, whereas in triploid representatives the chromosome number ranged from $3n = 141$ to $3n = 160$ (Cherfas 1966, Penaz et al. 1979, Fister and Soldatovic 1989, Fan and Shen 1990, Boroń 1994, Tóth et al. 2005).

The Prussian carp was introduced to Europe and to Poland from China and the Amur River (Sakai et al. 2009) at the beginning of the 20th century and now is widely distributed across lakes and rivers. Apart from two reports of an incidental occurrence of males within female populations (Skóra 1971, Rokicki and Kulikowski 1994) there are two papers containing respectively a chromosome number and morphology of the individuals from population of triploid females and diploids of both sexes (Boroń 1994, Boroń and Boroń 1996). Nevertheless, there has been no information on an occurrence of triploid male. We provide its chromosome number, some meristic characteristics, and reproductive potential by histology of gonad together with the data on other *C. gibelio* individuals of a certain age from two types of naturally existing unisexual triploid and a diploid-polyploid population.

This study deals with the ploidy level by karyotyping, some taxonomic characteristics by analysis of morphology and reproductive potential by histology of gonads of Prussian carp *C. gibelio* individuals of a certain age from two types of naturally existing unisexual triploid and a diploid-polyploid populations.

MATERIALS AND METHODS

In total 31 specimens of *Carassius gibelio* from three different populations inhabiting the Vistula River basin, Baltic Sea watershed were analyzed. Nineteen of them were collected in May and June 2005 from Lake Tały (53°50'N, 21°30'E), seven from the Siemianówka Dam Reservoir (52°55'N, 23°55'E) at the Narew River, and five from Lake Kisajno (54°10'N, 21°40'E).

The sex of all specimens was determined according to the morphology and histology of gonads. The peritoneum of all analyzed specimens was strongly pigmented as typical for *C. gibelio*. The number of annual increments of scales collected under the dorsal fin was used to age determination.

Chromosome preparation. Chromosome preparations were made according to the method described by Boroń (1994). Briefly, live fish were injected with a dose of 1 mL

of 0.05% colchicine solution per 100 g of body weight. After 1.5 h, the kidneys were removed and placed in a hypotonic solution of 0.075 M KCl for 30 min, then fixed in a solution of methanol and acetic acid with a ratio of 3 to 1. Spreads were made by dropping aforementioned cell suspension onto microscope slides. Air-dried slides were stained with 4% Giemsa solution. The chromosome classification of Levan et al. (1964) was adopted. At least 10 metaphase plates from each individual were analyzed.

Meristic and metric characteristics. The following meristic characteristics (external and internal) were analysed: soft (branched) fin ray numbers of: dorsal- (*D*), anal- (*A*), pectoral- (*P*), and ventral- (*V*) fins (two last branched dorsal and anal fin rays were counted as one); number of denticulations on the last unbranched dorsal and anal fin rays (*D*-serrae, *A*-serrae), number of lateral line (*ll*) scales, number of scales between lateral line and dorsal fin base (*ss*), number of scales between lateral line and ventral fin base (*i*), number of gill rakers on the first gill arc (*sp.br.*), number of pharyngeal teeth (*PhF*), and total number of vertebrae (*Vt*). Bilateral characters were counted on the left side of the body. The total number of vertebrae included four of Weberian apparatus. External counts were made in all 31 individuals, whereas vertebrae were counted from dry skeleton preparations of 17 specimens (Siemianówka Dam Reservoir: 7; Kisajno Lake: 5; Tały Lake: 5) obtained by boiling in hot water.

Twenty-three metric characteristics were analyzed following the methodology of Szlachciak (2000) (except for some abbreviations) and measured to the nearest 0.1 mm on the left side of the body: head length (*lc*), preorbital distance (*prO*), eye diameter (*O*), postorbital distance (*poO*), head depth (*hc*), head width (*lac*), lower jaw length (*lmd*), body (standard) length (BL), predorsal distance (*pD*), postdorsal distance (*poD*), maximum body depth (*H*), preanal distance (*pA*), minimum body depth (*h*), caudal peduncle length (*lpc*), body width (*laco*), pectoral fin length (*lP*), ventral fin length (*lV*), dorsal fin length (*lD*), anal fin length (*lA*), dorsal fin height (*hD*), anal fin height (*hA*), distance between pectoral and ventral fin (*P-V*), distance between ventral and anal fin (*V-A*).

The measurements were expressed as a percentage of the body (standard) length (BL) and head length (*lc*). All the data were statistically processed, involving means (\bar{x}) and standard deviations (SD) with the exception of the samples from the Siemianówka and Kisajno not representative for statistical analysis.

Histology of gonads. Histological analysis was done on the gonads of 19 triploid females from Lake Tały, two diploid females and five males (four diploid individuals and one triploid) from the Siemianówka Dam Reservoir, and of five triploid females from Lake Kisajno.

In order to determine the gonadosomatic index ($GSI = \text{gonad weight/body weight} \times 100$), ovaries and testes were weighed, to an accuracy of 0.01 g. The gonads were fixed in buffered formalin or in Bouin's solution, dehydrated and embedded in paraffin. Histological sections (7 μm) were stained with haematoxylin and eosin (HE).

The histological slides were used to determine the development stages of reproductive cells and gonads against the maturity scale of the ovaries of bony fishes, developed by Rinchar and Kestemont (1996). The names of oocytes development stages, marked with subsequent letters from A to E, were adopted after Juchno et al. (2007). The developmental stages of spermatogenesis were determined as described by Billard (1986).

RESULTS

Karyotype. All nineteen and five individuals of *Carassius gibelio* from Tałty and Kisajno Lakes respectively were triploid females possessing from 150 to 160 chromosomes plus from one to five additional microchromosomes. In most of the metaphase plates of *C. gibelio* from Tałty Lake 160 chromosomes were counted, including 4 microchromosomes. Karyotype was composed of 33 metacentrics, 48 submetacentrics, 75 subtelo-acrocentric chromosomes, and 4 microchromosomes (Figs. 1, 2). The karyotype (not presented here) of triploid females from Kisajno Lake was slightly different and frequently composed of 154 chromosomes, including two microchromosomes.

Among the specimens from the Siemianówka Dam Reservoir four males and two females showing $2n = 100$ chromosomes were found. Their modal karyotype was similar and composed of 13 metacentric, 19 submetacentric and 18 subtelo- to acrocentric chromosome pairs (Figs. 3, 4). Microchromosomes among the elements of the karyotypes of $2n = 100$ were not observed.

One male from the same locality was characterized by a modal number of 160 chromosomes, including 34 metacentric, 58 submetacentric, 62 subtelo-acrocentric, and 6 microchromosomes (Figs. 5, 6). The metaphases displayed variable numbers of chromosomes ranging from 151 to 162 (Table 1).

Meristic and metric characteristics. Data on meristic and metric characteristics of individuals of different ploidy level are given in Table 2. The individuals from Tałty Lake were characterized by lower mean values for the number of: soft rays in the dorsal fin, scales in lateral line and serrations in the dorsal and anal fins. The last unbranched dorsal and anal rays were thick, strongly ossified, and serrated in two rows along the posterior margin. The following formula can be used to describe the meristic characteristics of analyzed individuals of Prussian carp from all populations (ranges and modal values in parentheses): *D* 14–18 (16, 17, 18), *A* 5 (5), *P* 13–17 (14, 15, 16, 17), *V* 7–9 (7, 8), *II* 29–32 (30, 31, 32), *ss* 5–6 (6), *i* 5–7 (6), *sp. br.* 40–54 (46), *D-serrae* 7–17 (10, 11, 12, 14), *A-serrae* 10–21 (10, 11, 14, 15, 18), *PhF* 4–4, *Vt* 31–34 (32, 34).

Apart from the morphological features also the inner side of the abdominal cavity which was covered by black peritoneum in all of the investigated individuals was typical for *C. gibelio* species. Relative values of biometric characteristics from three analyzed samples are given in Table 3.

Histology of gonads. Among 19 triploid females collected in mid-May from Lake Tałty, ovaries of the largest

females, which body weight were from 13 to 23 g and were aged 3 years, were in the IV stage of maturity. The ovaries contained mainly oocytes with yolk (stage E), oocytes in the cortical alveolus stage (C, D), and fewer previtellogenic oocytes (B) (Fig. 7). The value of the gonadosomatic index was low and ranged from 6% to 8%. The ovaries of the smallest females (the age of 2) were in the II stage of maturity and were filled with previtellogenic oocytes (B) (Fig. 8). The GSI was very low and amounted to 1% or 2%. The ovaries of remaining *C. gibelio* from Lake Tałty (the age of 2 or 3) were in the III stage of maturity. They contained mainly oocytes in the cortical alveolus stage (C, D) (Fig. 9). The value of the GSI in these females ranged from 3% to 7%.

In the ovaries of two diploid females with body weight of about 700 g and the age of 8, collected on June 10 from the Siemianówka Dam Reservoir, postovulatory follicles (f) were observed, indicating that a batch of eggs had been laid. The ovaries contained vitellogenic oocytes as well as oocytes in the cortical alveolus stage (C, D), and oocytes accumulating yolk (E), also atretic oocytes (Fig. 10). Such histology of gonads is typical of stage IV₂. The GSI ranged from 6% to 7%.

Three triploid females (aged 3 and with a body weight from 102 to 205 g), captured in Lake Kisajno in mid-June, had laid the first batch of eggs and their ovaries were in IV₂ or III₂ stage (Fig. 11). Moreover the ovaries of two triploid females were in V stage of maturity (before spawning) with oocytes with merged yolk granules (Fig. 12). The GSI ranged from 7% in the smallest female to 16% in the biggest one.

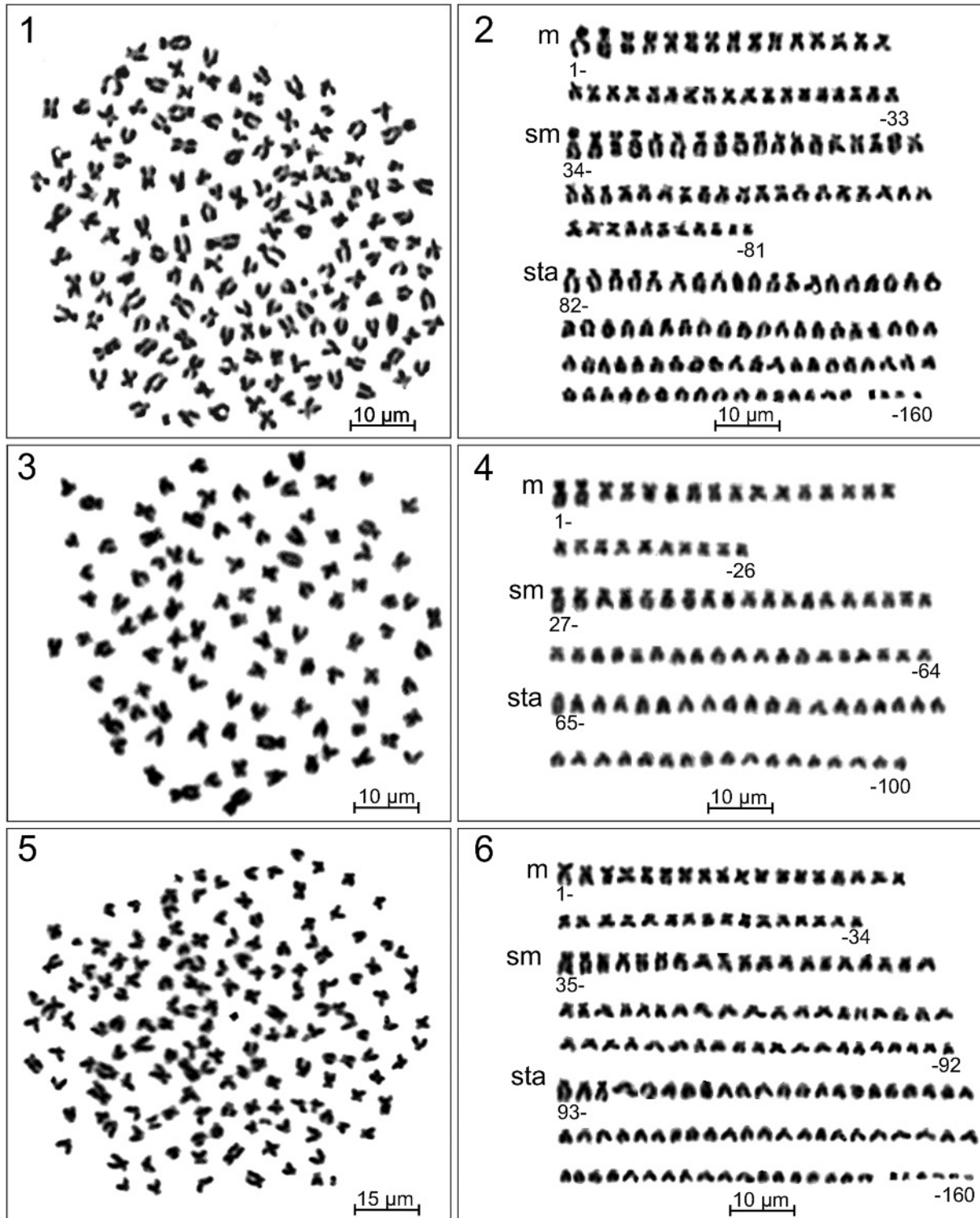
The testes of diploids and one triploid male in June in the Siemianówka Dam Reservoir showed typical morphological features. Seminal tubules with cysts filled with germ cells at various developmental stages, i.e. spermatogonia, spermatocytes, spermatids, and spermatozoa, were observed in both diploid and triploid males. The testis of the biggest diploid male (at age of 8) was filled mainly by spermatozoa. In the testes of other diploid individuals (Fig. 13) and one triploid (Fig. 14), which were at age of 7, spermatozoa dominated in the tubules but spermatocytes I, spermatocytes II and spermatids were adhering to the seminal tubule sheaths. The GSI of diploids ranged from 2 to 3% and of triploid individual was 1.5%.

DISCUSSION

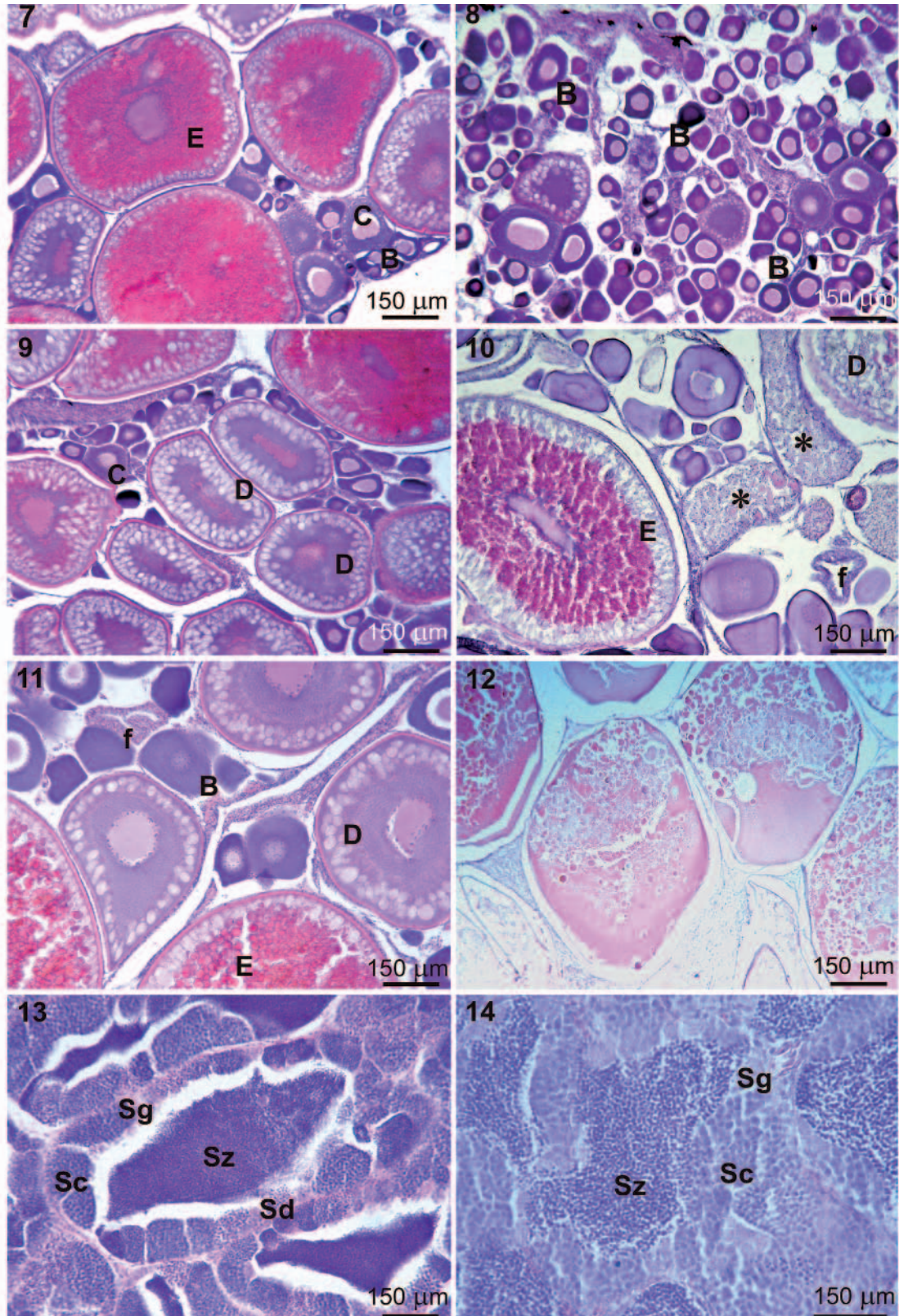
Karyotype. The karyotype data of *Carassius gibelio* summarized in Table 1 revealed the same number $2n = 100$ chromosomes of diploid individuals (Table 1). Formerly diploid males and females of *C. gibelio* were identified together with triploid females in the Zegrzynski Dam Reservoir, at the Vistula River basin (Boroń 1994). The substantial differences in the chromosome structure of triploids (Table 1) are typical for polyploid cyprinids with relatively large number but small sized chromosomes. Variable numbers of supernumerary microchromosomes in the karyotype of triploid individuals, including artificially produced clones have been found (Zhou

and Gui 2002, Yi et al. 2003). Similarly, different number of supernumerary microchromosomes in the karyotype of allogynogenetic offspring of the Amazon molly *Poecilia formosa* was observed (Schartl et al. 1995). The origin of these microchromosomes remains uncertain. They were thought to originate from the genome of the host species

(Schartl et al. 1995). The experiments with painting probe prepared from the microdissected microchromosomes indicated that some paternal chromosomes fragments have been incorporated into the genome of the host species (Yi et al. 2003). Karyotypic diversity might be correlated with diploidization as a subsequent process



Figs. 1–6. Metaphase plates (on the left) and karyotypes (on the right) of Prussian carp, *Carassius gibelio*; **Figs. 1, 2.** Triploid female ($3n = 160$) from Lake Tałty; **Figs. 3, 4.** Diploid male ($2n = 100$) from Siemianówka Dam Reservoir; **Figs. 5, 6.** Triploid male ($3n = 160$) from Siemianówka Dam Reservoir; Abbreviations: m, meta-centric; sm, submetacentric; sta, subtelo- and acrocentric chromosomes



Figs. 7–14. Cross-section of ovaries (Figs. 7–12) and testes (Figs. 13–14) of Prussian carp, *Carassius gibelio* during the reproductive period; **Fig. 7.** Ovary in IV maturity stage (Lake Tałty, May); **Fig. 8.** Ovary in II maturity stage (Lake Tałty, May); **Fig. 9.** Ovary in III maturity stage (Lake Tałty, May); **Fig. 10.** Ovary in IV₂ maturity stage (Siemianówka Reservoir, June); **Fig. 11.** Ovary in IV₂ maturity stage (Lake Kisajno, June); **Fig. 12.** Ovary in V maturity stage (Lake Kisajno, June); **Fig. 13.** Testis of diploid male (Siemianówka Reservoir); **Fig. 14.** Testis of triploid male (Siemianówka Reservoir); Abbreviations: B, C, D, E, stages of oocytes development; f, postovulatory follicles; * atretic oocytes; Sc, cysts of spermatocytes and Sg, spermatogonia; Sd, spermatids; Sz, spermatozoa

after polyploidization to achieve stability of the genome (Zhou and Gui 2002). The different number of chromosomes also reported in the present study might be correlated with the hybrid origin of some *C. gibelio* clones. This species easily hybridize with the common carp *Cyprinus carpio* or *Carassius carassius* (see Zhou and Gui 2002, Tóth et al. 2005).

In the present study the triploid male from Polish waters is described for the first time. However, fertile triploid males ($3n = 156$) of this species with normal morphology of germ cells at all stages of gametogenesis were found in natural triploid gynogenetic populations in north-east China (Shen et al. 1983, Fan and Shen 1990, Zhou et al. 2000, Yang et al. 2008). Later triploid *C. gibelio* males ($3n = 130$ – 150) were found in the Lower Don drainage (Abramenko et al. 1997), Sea of Azov basin (Abramenko et al. 2004) and in the Dyje River, Czech Republic (Flajšhans et al. 2008).

So, in the Vistula River basin triploid Prussian carp females can be also produced by a gonochoristic mode of reproduction with triploid males as described by Zhou et al. (2000). Some of them originated via gynogenesis or allogynogenesis, when heterologous sperm from other fish species, apart from activating egg and embryo development, is able to contribute to the phenotype of the gynogenetic offspring. High chromosomal diversity within

triploid *C. gibelio* females distributed in the Vistula River basin might be associated to their different origin and further molecular studies on naturally and experimentally produced progenies are necessary to explain these complicated processes.

Meristic and metric characteristics. Compared to the samples from the Siemianówka Reservoir and Kisajno Lake, the analyzed Prussian carp from Tałty Lake was characterized by lower mean values for the number of soft rays in the dorsal fin, the number of lateral line scales and the number of serrations in the dorsal and anal fins.

The analyzed fish were similar to those given by other researchers as regards the ranges and mean values of external meristic characteristics. Some differences were observed in the number of vertebrae (Table 2), probably caused by different methods of counting the vertebrae that are not explained in most papers.

The analyzed fishes displayed the pharyngeal teeth formula 4–4. The same pattern was given by Skóra (1971), Rokicki and Kulikowski (1994), and Boroń and Boroń (1996).

There are some data in the literature concerning the morphological differences connected with the ploidy level. Significant differences between diploid and triploid populations of *C. gibelio* were found in some metric and meristic characteristic as the number of lateral line scales

Table 1

Karyotype data on the Prussian carp, *Carassius gibelio*, from different localities

Karyotype	NF	Sex	Locality	Reference
$2n = 94$ $3n = 141$		F, M F	Byelorussia	Cherfas (1966)
$3n = 150$		F	Danube, Bosnia	Sofradžija et al. (1978)
$3n = 160$ $46m + 82sm, st + 32a$		F	River Dyje, Danube basin	Penaz et al. (1979)
$3n = 160$ $16m + 28sm + 126sta$		F	Danube basin Yugoslavia	Vujošević et al. (1983)
$3n = 158$ $36m + 54sm, st + 68a$		F	Pancevacki Rit, Yugoslavia	Fister and Soldatovic (1989)
$3n = 156$ $42m + 74sm + 40st$		F, M	North-eastern China	Fan and Shen (1990)
$2n = 100$ $14m + 24sm + 62sta$	138	F, M	Zegrzyński Reservoir, Vistula	Boron (1994)
$3n = 150$ $26m + 50sm + 74sta$	226	F	River	
$3n = 156$ $36m + 54sm + 36st + 24a + 6$	240	F	Northern China	Zhou and Gui (2002)
$3n = 162$ $42m + 54sm + 36st + 24a + 6$	252	F	Pengze Lake, Jiangxi, China	
$3n = 154$ $24m + 54sm + 72sta + 4$	228	F	Kisajno Lake	Presently reported study
$3n = 160$ $33m + 48sm + 75sta + 4$	237	F	Tałty Lake	
$2n = 100$ $26m + 38sm + 36sta$	164	M, F	Siemianówka Reservoir	
$3n = 160$ $34m + 58sm + 62sta + 6$	246	M		

F, females; M, males; m, metacentric; NF, number of chromosome arms; sm, submetacentric; st, subtelocentric; sta, subtelocentric to acrocentric chromosomes.

and gill rakers on the first gill arch and selected indexes of the skull measurements (Vasil'eva 1990, Vasil'eva and Vasilev 2000, Mezhrherin and Kokodii 2008).

An occurrence of males in European populations of *C. gibelio* is rare so it was not possible to discuss differences between sexes. One male with very poorly developed gonads was found among 96 individuals from Golysz Ponds (Skóra 1971) and another one in Lake Karas, near Gdansk (Rokicki and Kulikowski 1994). According to Nikol'skij (1956) males were slightly smaller than females, had less number of gill rakers, a longer and lower caudal peduncle, a shorter and lower dorsal fin, a lower anal fin and shorter pectoral fins. Rolik and Rembiszewski (1987) described the sexual dimorphism only in lengths of paired fins and caudal peduncle which were bigger in males. The study of 68 females and 14 males collected from the Zegrzynski Dam Reservoir (Boroń and Boroń 1996) did not confirm the above-mentioned differences between sexes.

Differences observed in morphological features among samples from the three populations may be attributed to the differences in body length and age, and to environmental conditions.

Reproduction. The obtained results revealed that diploid and triploid individuals of Prussian carp spawn in batches and they have an asynchronous ovary, which contains oocytes in all the developmental stages. Postovulatory follicles, which indicated that the first batch of eggs had been laid, were observed in mid-June in both diploids and triploids.

A histological analysis of the ovaries indicated the Prussian carp (triploids) were mature at the age of 3. The ovaries of females (at the age of 3) from Lake Tałty or from Lake Kisajno were in the IV or IV₂, and III₂ stage of

maturity, respectively, contained mainly oocytes with yolk (exogenous vitellogenesis) and oocytes in the cortical alveolus stage (endogenous vitellogenesis). The body length of the smallest matured female from Lake Tałty was only 69 mm. In the lakes of Turkey females of Prussian carp achieve maturity at three years of age, which had body length of 80 mm (Balık et al. 2004) or 230.80 mm (Şaşı 2008).

The results of this study confirm that taking into account histological structure of gonads diploid and triploid (one) males of Prussian carp might reproduce. Spermatozoa observed in their testes seem to be able to fertilize bisexual eggs or stimulated gynogenetic eggs. The testis of triploid male cannot be morphologically distinguished from the testes of diploids, but the gonad weight of triploid Prussian carp male was clearly lower than testes of diploids of the same body weight. Variability in the number of chromosomes in the triploid male from the Siemanówka Dam Reservoir may indicate the aneuploid nature of the sperm. Triploids and one tetraploid male were found to produce sperm with highly variable DNA content (Flajšhans et al. 2008). Data on spermiogenesis in artificially induced triploids (Pandian and Koteeswaran 1998) revealed that the mobility of aneuploid spermatozoa of triploid males was lower than that of euploid cells. Spermatozoa viability of diploid and triploid males was higher than that of the tetraploid, however the percentage of motile spermatozoa in triploids was significantly lower in comparison with tetraploids and diploids (Flajšhans et al. 2007, 2008). So, further experimental studies on fertilization ability of the sperm of triploid males are required.

Prussian carp might reproduce both gynogenetically and gonochoristically and might produce two kinds of eggs:

Table 2
Values of external and internal meristic characters of Prussian carp, *Carassius gibelio*, from three populations

Feature	Tałty Lake			Siemanówka Reservoir				Kisajno Lake		
	3n F, n = 19			2n F, n = 2		2n M, n = 4		3n M, n = 1	3n F, n = 5	
	Range	\bar{x}	SD	Range	\bar{x}	Range	\bar{x}		Range	\bar{x}
<i>D</i>	14–18	15.6	0.90	16–17	16.5	15–18	17.0	17.0	16–18	17.0
<i>A</i>	5.0	5.0	0.00	5.0	5.0	5.0	5.0	5.0	5.0	5.0
<i>P</i>	14–16	14.7	0.58	14–16	15.0	13–17	15.0	15.0	13–17	15.4
<i>V</i>	7–9	7.7	0.57	7–8	7.5	7–9	8.0	7.0	7–9	7.8
<i>ll</i>	29–31	30.4	0.61	30–32	30.8	29–32	30.9	32.0	31–32	31.4
<i>ss</i>	5–6	5.7	0.48	6.0	6.0	6.0	6.0	6.0	6.0	6.0
<i>i</i>	5–7	6.2	0.50	6.0	6.0	6.0	6.0	6.0	5–6	5.8
<i>(sp.br.)</i>	39–49	42.9	2.95	45–54	49.5	40–47	43.5	39.0	–	–
<i>D-serrae</i>	7–11	9.6	1.24	13–17	15.0	14–16	14.7	12.0	11–17	13.0
<i>A-serrae</i>	10–15	11.1	1.37	17–21	19.0	18–22	20.0	18.0	14–15	14.5
<i>Vt</i>	31–32	31.6	0.55	32.0	32.0	32–33	32.5	33.0	33–34	33.6

F = females; M = males; \bar{x} = mean; n = number of individuals; SD = standard deviation; Meristic characters (number of...): *D* = soft rays in dorsal fin; *A* = soft rays in anal fin; *P* = soft rays in pectoral fin; *V* = soft rays in ventral fin; *ll* = scales in the lateral line; *ss* = scales between lateral line and dorsal fin base; *i* = scales between lateral line and ventral fin base; *sp.br.* = gill rakers on the first gill arch; *D-serrae* = denticulations; *A-serrae* = denticulations; *Vt* = vertebrae (total No.).

Table 3

Relative values of biometric characters of Prussian carp, *Carassius gibelio*, from three populations

Character	Tały Lake			Siemianówka Reservoir			Kisajno Lake		
	3n F, n = 19			2n F, n = 2			3n M, n = 1		
	Range	\bar{x}	SD	Range	\bar{x}	SD	Range	\bar{x}	SD
BL [cm]	5.6–8.16	6.8	7.43	28.60–29.10	27.95	7.43	24.80–28.70	26.68	7.43
Head length <i>lc</i>	28.7–31.9	30.2	0.95	24.95–25.63	25.29	0.95	25.08–25.81	25.45	0.95
Predorsal distance <i>pD</i>	52.9–59.4	56.5	1.77	48.25–51.27	49.76	1.77	48.71–52.92	50.59	1.77
Postdorsal distance <i>po</i>	22.0–27.9	24.3	1.42	25.90–27.01	26.45	1.42	23.11–26.04	24.82	1.42
Maximum body depth <i>H</i>	38.1–47.5	42.8	3.13	38.35–43.92	41.13	3.13	39.44–42.30	40.96	3.13
Prealanal distance <i>pA</i>	70.0–80.2	73.8	2.51	70.45–74.85	72.65	2.51	71.08–75.66	73.06	2.51
Minimum body depth <i>h</i>	12.8–14.8	13.8	0.59	16.56–18.10	17.33	0.59	15.92–17.70	16.92	0.59
Caudal peduncle length <i>lpc</i>	12.5–18.4	15.4	1.67	17.13–17.77	17.45	1.67	14.18–18.11	16.44	1.67
Body width <i>laco</i>	14.0–18.1	15.7	1.16	14.95–19.33	17.14	1.16	14.72–17.63	15.97	1.16
Pectoral fin length <i>lP</i>	18.1–21.7	20.3	1.02	17.59–19.70	18.65	1.02	19.48–22.14	20.65	1.02
Ventral fin length <i>lV</i>	19.6–26.9	22.5	1.90	19.11–21.23	20.17	1.90	22.26–23.56	22.99	1.90
Dorsal fin height <i>hD</i>	19.4–26.1	22.8	2.10	15.53–19.51	17.52	2.10	16.53–19.62	18.53	2.10
Anal fin height <i>hA</i>	12.9–20.6	17.3	2.20	13.68–15.52	14.60	2.20	14.96–16.44	15.76	2.20
Dorsal fin length <i>lD</i>	28.1–35.3	32.0	2.19	36.08–36.15	36.11	2.19	35.66–40.77	37.55	2.19
Anal fin length <i>lA</i>	8.4–12.5	10.2	1.01	10.19–10.48	10.33	1.01	11.25–12.68	11.76	1.01
P–V distance	19.1–25.7	21.5	1.70	18.87–20.22	19.54	1.70	19.70–21.39	20.54	1.70
V–A distance	24.3–34.0	30.2	2.77	30.34–32.13	31.23	2.77	29.85–35.66	32.13	2.77
<i>lc</i> [cm]	1.8–2.5	2.1	2.28	6.87–7.26	7.07	2.28	6.31–7.20	6.79	2.28
Preorbital distance <i>prO</i>	22.5–29.2	24.2	1.75	25.90–27.66	26.78	1.75	26.18–27.26	26.57	1.75
Eye diameter <i>O</i>	23.3–29.9	27.0	1.79	18.46–18.63	18.54	1.79	18.24–20.29	18.86	1.79
Postorbital distance <i>poO</i>	45.6–56.4	52.5	3.00	55.79–56.77	56.28	3.00	54.58–61.03	57.91	3.00
Head depth <i>hc</i>	83.5–99.5	91.4	4.43	88.98–92.14	90.56	4.43	88.89–99.21	93.01	4.43
Head width <i>lac</i>	47.2–64.1	54.8	4.82	57.85–62.15	60.00	4.82	59.31–63.01	62.01	4.82
Lower jaw length <i>lmd</i>	27.5–42.7	33.5	3.57	36.39–37.88	37.13	3.57	36.26–38.09	39.97	3.57

 \bar{x} , mean; F, females; M, males; n, number of individuals; SD, standard deviation. BL, body (standard) length.

“G” (gynogenetic) and “B” (bisexual) type eggs. The first type of eggs constitute about 50%–90% of the eggs, but the second about 10%–50% of all eggs (Fan and Liu 1990, Fan and Shen 1990, Zhou et al. 2000). The ratio of “G” to “B”-type eggs might be influenced by environmental conditions (temperature, hydrostatic pressure) or by age, health, and nutritional condition of the fish (Fan and Shen 1990). The results of chromosome and RAPD analysis demonstrate that diploid females reproduce sexually with males of Prussian carp and other cyprinids and the offspring of intra- and interspecific crosses contain the paternal DNA (Tóth et al. 2005). Triploid females usually reproduced by gynogenesis and their offspring were clones, but very rare paternal genes were actually transmitted to the offspring and the progeny were triploid interspecific hybrids (Tóth et al. 2005).

ACKNOWLEDGEMENTS

This work was carried out within the project No. 0208.805 of the University of Warmia and Mazury in Olsztyn, Poland. Partially, the study has been done under collaboration within the international project “Cypriniformes Tree of Life”, National Science Foundation # EF-0431326, coordinated by Dr R. Mayden. The study was conducted with the consent from the local Ethics Commission; individual consent no. 20/01.

REFERENCES

- Abramenko M.I., Kravchenko O.V., Velikoivanenko A.E.** 1997. Population genetic structure of the goldfish *Carassius auratus gibelio* diploid-triploid complex from the Don River Basin. *Journal of Ichthyology* **37** (1): 62–71.
- Abramenko M.I., Nadtoka E.V., Makhotkin M.A., Kravchenko O.V., Poltavtseva T.G.** 2004. Distribution and cytogenetic features of triploid males of crucian carp in Azov Basin. *Russian Journal of Developmental Biology* **35** (5): 305–315. DOI: 10.1023/B:RUDO.0000043825.97296.54.
- Balik İ., Özkök R., Çubuk H., Uysal R.** 2004. Investigation of some biological characteristics of the silver crucian carp, *Carassius gibelio* (Bloch 1783) population in Lake Eğirdir. *Turkish Journal of Zoology* **28** (1): 19–28.
- Billard R.** 1986. Spermatogenesis and spermatology of some teleost fish species. *Reproduction, Nutrition, Development* **26** (4): 877–920. DOI: 10.1051/rnd:19860601.
- Boroń A.** 1994. Karyotypes of diploid and triploid silver crucian carp *Carassius auratus gibelio* (Bloch). *Cytobios* **80**: 117–124.
- Boroń A., Boroń S.** 1996. Meristic and biometric parameters of silver crucian carp *Carassius auratus gibelio* (Bloch, 1783) from Zegrzyński Dam Reservoir. *Acta Academiae Agriculturae Ac Technicae Olstenensis, Protectio Aquarum et Piscatoria* **22**: 3–13.
- Cherfas N.B.** 1966. Natural triploidy in females of the unisexual form of silver crucian carp (*Carassius auratus gibelio* Bloch). *Genetika* **2** (5): 16–24.
- Devlin R.H., Nagahama Y.** 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* **208** (3–4): 191–364. DOI: 10.1016/S0044-8486(02)00057-1.
- Fan Z., Liu G.** 1990. The ploidy and reproductive mechanism of crucian carp, *C. auratus gibelio*. *Journal of Fish Biology* **36** (3): 415–419. DOI: 10.1111/j.1095-8649.1990.tb05621.x
- Fan Z., Shen J.** 1990. Studies on the evolution of bisexual reproduction in crucian carp (*C. auratus gibelio* Bloch). *Aquaculture* **84** (3–4): 235–244. DOI: 10.1016/0044-8486(90)90089-6.
- Fister S., Soldatovic B.** 1989. Karyotype analysis of a gynogenetic population of *Carassius auratus gibelio* Bloch (Cyprinidae) from Pancevacki Rit [Yugoslavia]. *Acta Veterinaria* **39** (5–6): 259–267.
- Flajšhans M., Rodina M., Halačka K., Vetešník L., Gela D., Lusková V., Lusk S.** 2008. Characteristics of sperm of polyploid Prussian carp *Carassius gibelio*. *Journal of Fish Biology* **73** (1): 323–328. DOI: 10.1111/j.1095-8649.2008.01937.x.
- Flajšhans M., Rodina M., Halačka K., Vetešník L., Papošuek I., Gela D., Lusková V., Lusk S.** 2007. Ploidy levels and reproductive patterns of *Carassius auratus gibelio* as revealed by means of artificial hybridization and flow cytometry. *Conference Information: 9th International Symposium on Genetics in Aquaculture. Aquaculture* **272** (Suppl. 1): S256. DOI: 10.1016/j.aquaculture.2007.07.059.
- Juchno D., Boroń A., Golaszewski J.** 2007. Comparative morphology and histology of the ovaries of the spined loach *Cobitis taenia* L. and natural allopolyploids of *Cobitis* (Cobitidae). *Journal of Fish Biology* **70** (5): 1392–1411. DOI: 10.1111/j.1095-8649.2007.01419.x.
- Levan A., Fredga K., Sandberg A.A.** 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* **52** (2): 201–220. DOI: 10.1111/j.1601-5223.1964.tb01953.x.
- Mezhzherin S.E., Kokodii S.V.** 2008. Genetic homeostasis and developmental stability in natural populations of bisexual (*Carassius auratus*) and unisexual (*C. gibelio*) goldfishes. *Cytology and Genetics* **43** (5): 336–342. DOI: 10.3103/S0095452709050077.
- Nikol'skij G.V. [Nikolsky G.V.]** 1956. *Ryby bassejna Amura. [Fishes of the Amur River basin.]* Izdatel'stvo Akademii Nauk SSSR, Moskva. [In Russian.]
- Pandian T., Koteeswaran J.R.** 1998. Ploidy induction and sex control in fish. *Hydrobiologia* **384** (1–2): 167–243. DOI: 10.1023/A:1003332526659.
- Penaz M., Ráb P., Prokeš M.** 1979. Cytological analysis, gynogenesis and early development of *Carassius auratus gibelio*. *Acta Scientiarum Naturalium Brno* **13** (7): 1–33.
- Rinchard J., Kestemont P.** 1996. Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and histological features. *Journal of Fish Biology* **49** (5): 883–894. DOI: 10.1111/j.1095-8649.1996.tb00087.x.
- Rokicki J., Kulikowski M.** 1994. Występowanie w Polsce samców karasia srebrzystego *Carassius auratus gibelio* (Bloch, 1783). [Occurrence of male *Carassius auratus gibelio* (Bloch, 1783) in Poland.] *Przegląd Zoologiczny* **38** (1–2): 89–92. [In Polish.]
- Rolik H., Rembiszewski J.M.** 1987. *Ryby i Kręglouste. [Fishes and cyclostomes.]* PWN, Warszawa. [In Polish.]
- Sakai H., Iguchi K., Yamazaki Y., Sideleva V.G., Goto A.** 2009. Morphological and mtDNA sequence studies on three crucian carps (*Carassius*: Cyprinidae) including a new stock from the Ob River system, Kazakhstan. *Journal of Fish Biology* **74** (8): 1756–1773. DOI: 10.1111/j.1095-8649.2009.02203.x.

- Schartl M., Nanda I., Schlupp I., Wilde B., Epplen J.T., Schmid M., Parzefall J.** 1995. Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* **373**: 68–71. DOI: 10.1038/373068a0.
- Shen J., Wang G., Fan Z.** 1983. The ploidy of crucian carp and its geographical distribution in Heilongjiang main river. *Journal of Fisheries of China* **7** (2): 87–94. [Abstract in English, text in Chinese.]
- Skóra S.** 1971. The German carp (*Carassius auratus gibelio* Bloch) from the river Iłownica stocked in a carp pond. *Acta Hydrobiologica* **13** (2): 217–232.
- Sofradžija A., Berberović L.J., Hadziselimović R.** 1978. Chromosome sets of *Carassius carassius* and *Carassius auratus gibelio*. *Acta Biologica Jugoslavica* **E 10**: 135–43.
- Szlachciak J.** 2000. Kształt ciała, ubarwienie, cechy biometryczne i merystyczne. [Body shape, colouration, biometric and meristic characters.] Pp. 49–68. *In*: Brylinska M. (ed.) *Ryby słodkowodne Polski*. [The freshwater fishes of Poland.] PWN, Warszawa. [In Polish.]
- Şaşı H.** 2008. The length and weight relations of some reproduction characteristics of Prussian carp, *Carassius gibelio* (Bloch, 1782) in the South Aegean Region (Aydn-Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* **8** (1): 87–92.
- Tóth B., Várkonyi E., Hidas A., Edviné Meleg E., Váradi L.** 2005. Genetic analysis of offspring from intra- and interspecific crosses of *Carassius auratus gibelio* by chromosome and RAPD analysis. *Journal of Fish Biology* **66** (3): 784–797. DOI: 10.1111/j.0022-1112.2005.00644.x.
- Vasil'eva E.D. [Vasilyeva Ye.D.]** 1990. O morfoložičeskoj divergencii ginogenetičeskoj i biseksual'noj form serebrânogo karasâ *Carassius auratus* (Cyprinidae, Pisces). [On morphological divergence of gynogenetic and bisexual forms of *Carassius auratus* (Cyprinidae, Pisces). *Zoologičeskij Žurnal [Zoologičesky Zhurnal]* **69** (11): 97–110. [In Russian.]
- Vasil'eva E.D., Vasil'ev V.** 2000. The origin and taxonomic status of the triploid form of the goldfish, *Carassius auratus* (Cyprinidae). *Journal of Ichthyology* **40** (8): 553–563.
- Vujošević M., Živković S., Rimsa D., Jurišić S., Cakić P.** 1983. The chromosomes of nine fish species from the Dunav basin in Yugoslavia. *Acta Biologica Jugoslavica; Ichthyologia* **E 15** (2): 29–40.
- Yang J., Chen W., Wu H., Fan Z.** 2008. Comparative study on the spermary histological structure of triploid and diploid *Carassius auratus gibelio*. *Journal of Fisheries of China* **32** (1): 21–26. [Abstract in English, text in Chinese.]
- Yi M.S., Li Y.Q., Liu J.D., Zhou L., Yu Q.X., Gui J.F.** 2003. Molecular cytogenetic detection of paternal chromosome fragments in allogynogenetic gibel carp, *Carassius auratus gibelio* Bloch. *Chromosome Research* **11** (7): 665–671. DOI: 10.1023/A:1025985625706.
- Zhou L., Gui J.F.** 2002. Karyotype diversity in polyploid gibel carp, *Carassius auratus gibelio* Bloch. *Genetica* **115** (2): 223–232. DOI: 10.1023/A:1020102409270.
- Zhou L., Wang Y., Gui J.F.** 2000. Analysis of genetic heterogeneity among five gynogenetic clones of silver crucian carp, *Carassius auratus gibelio* Bloch, based on detection of RAPD molecular markers. *Cytogenetics and Cell Genetics* **88** (1–2): 133–139. DOI: 10.1159/000015506.

Received: 17 March 2010

Accepted: 22 September 2010

Published electronically: 25 March 2011