Composition and characteristic of subadult water frogs sample (Pelophylax esculentus complex)

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Composition and characteristic of subadult water frogs sample (*Pelophylax esculentus* complex)

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Mass polyploidy in *Pelophylax esculentus* was discovered in the middle part of Siverskyi Donets River basin in eastern Ukraine. This territory proved to be unique in terms of *P. esculentus* complex population systems variety, and was named Siverskyi Donets center of green frogs' diversity. Population systems here consist of *P. ridibundus* and both diploid and triploid *P. esculentus*. The latter is interspecific hybrid, which reproduce hemiclonally by crossing with parental species, *P. ridibundus* or *P. lessonae* (Plötner, 2005). The majority of water frogs' population systems investigation deals with samples of mature animals, while subadults are less studied.

We collected the random sample of 73 small water frogs from the Siverskyi Donets River floodplain in Kharkiv Oblast of Ukraine in September 2014. All frogs were measured, injected with colchicines and sacrificed after anesthesia. We collected blood smears, intestine and testes for karyological analysis, and second toes for age determination.

Frogs ranged in length from 20,7 to 45,3 mm and aged 1 to 5 years. The sample included 34 females and 39 males. We defined 10 females as *P. esculentus* (5 diploids and 5 triploids) and 24 as *P. ridibundus*. There were 26 *P. esculentus* (20 diploids and 6 triploids) and 13 *P. ridibundus* among males. As expected, the erythrocytes of triploids were significantly larger than diploids' ones (Fig. 1). Although the boundary revealed was about 28 micron, we observed overlapping of diploid and triploid cells size. Therefore in such borderline cases true ploidy could be determined by karyological technique only. We found, that there is significant positive correlation between erythrocyte size and body length in both diploids and triploids. Spearman correlation coefficient reached 0.72 (p=0.013) in diploid hybrids and *P. ridibundus*, while in triploids this coefficient was 0.30 (p=0.018).

Average ratio between testes length and body length appeared to be larger in parental species than in both diploid and triploid hybrids (Fig. 2).

Age was determined by skeletochronology technique. The majority of individuals were 2 (52%) and 3 years old (22%). Based on the correlation between body length and age we have divided the subadults into three groups: precocious, stunted and undefinable (Fig. 3). We suggest that these groups represent different ontogenetic strategy (Шабанов и др., 2014).

Rare mature sperms were observed in testes of diploid *P. esculentus* that reached 4 and 5 years. However, among triploids and *P. ridibundus* there were males aged only two years, which produced a few sperm. Further karyological analysis revealed that only about 6% of spermatocytes I in diploid *P. esculentus* testes had normal karyotype with 13 bivalents (Fig. 4). The majority of the meiotic cells have diploid chromosome set that did not form normal bivalents (37%; Fig. 5, B), while about 6% had doubled number of chromosome and the rest were haploid or aneuploid (Fig. 5, D). In total, 169 meiotic plates were analysed. The proportion of cells of different ploidy varied greatly among individuals (Fig. 6). Triploid hybrid males had no normal spermatocytes I with 13 bivalents at all (53 plates analysed; Figs. 4 and 7). About 11% of meiotic divisions included 39 (3n) chromosomes, which were composed in bivalents, multivalents and univalents (Fig. 5, C). These cells probably resulted from normal 3n spermatogonia, which entered meiosis without elimination of one parental genome. *P. ridibundus* demonstrated more stable gametogenesis (Figs. 4 and 8) and had 54% of diploid spermatocytes with 13 bivalents (Fig. 5, A; 99 plates analysed). Among mitotic spermatogonial plates we counted 25% of normal diploid cells in *P. ridibundus* and 31% of such in diploid *P. esculentus*. This proportion was much lower in the triploids' testes, reaching only 5%, while the number of 3n mitotic plates was higher (11%; Fig. 9). In total, 126 mitotic plates were analyzed in *P. ridibundus*, 297 in diploid *P. esculentus* and 56 in triploid *P. esculentus*.

These results allow us to conclude that diploid and triploid *P. esculentus* have considerably disturbed cell division and, probably, constrained development of gonads.

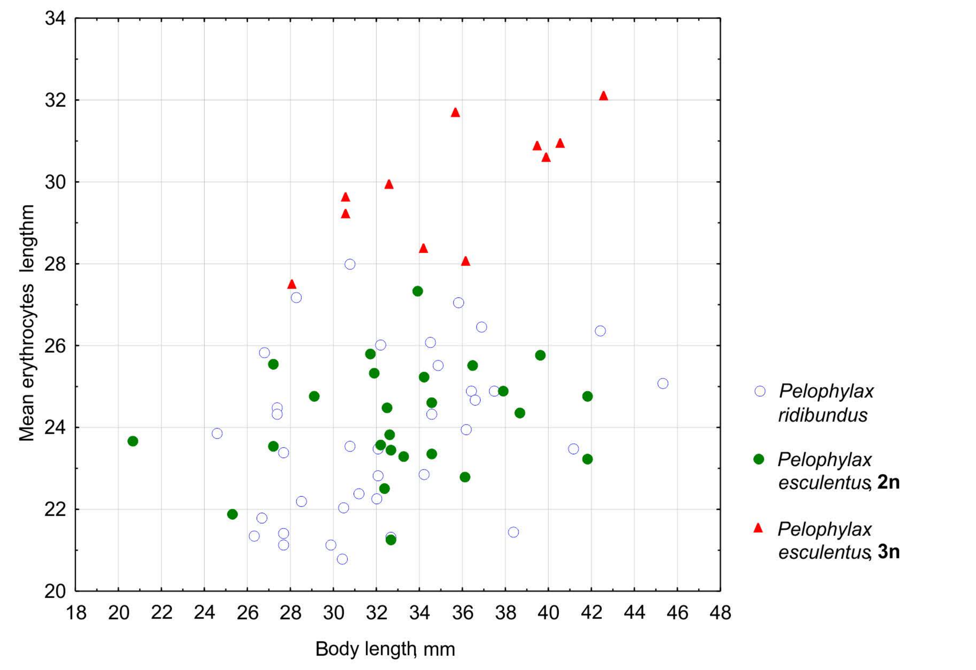

Fig. 1. Relation of mean erythrocyte length to the body length. *P. ridibundus* are designated by blue open circles, diploid *P. esculentus* by green circles, triploid *P. esculentus* by red triangles

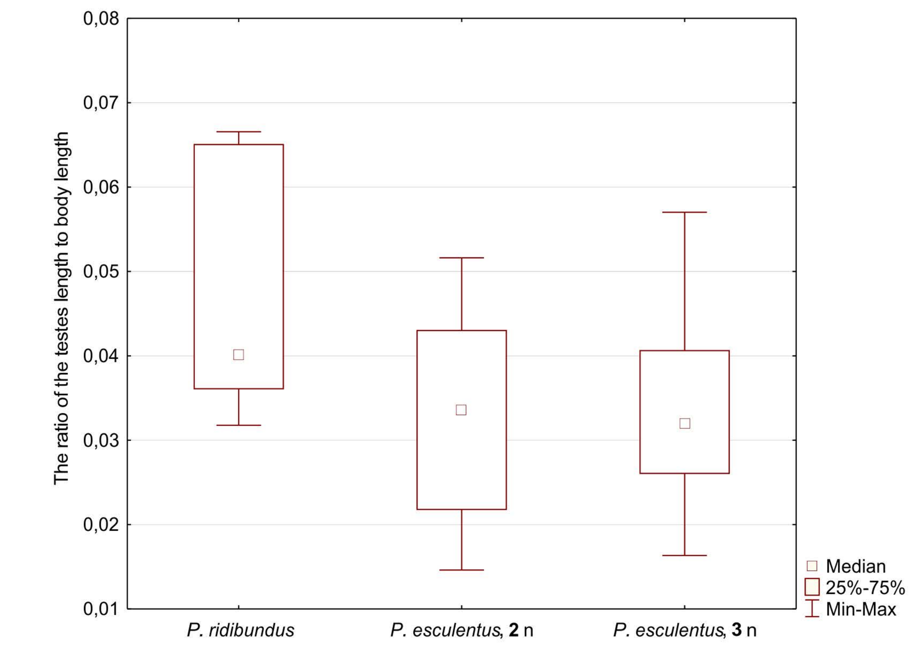

Fig. 2. Values of ratio between the testes length and the body lentgh in *P. ridibundus*, diploid *P. esculentus* and triploid *P. esculentus*

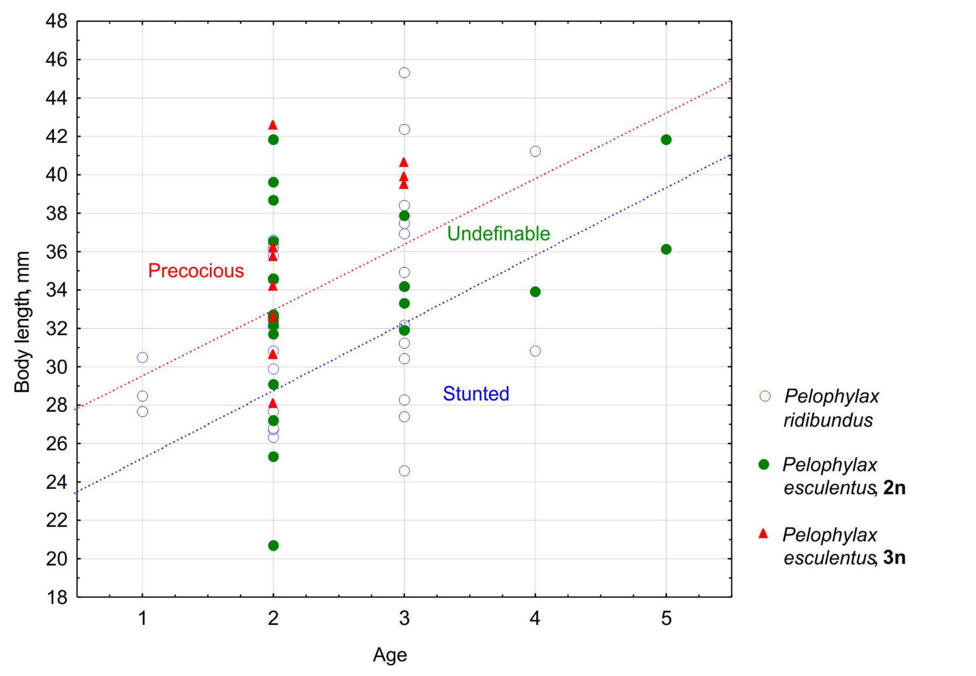

Fig. 3. Correlation between frogs' body length and their age. Three groups of individuals, which indicatively represent different ontogenetic strategies

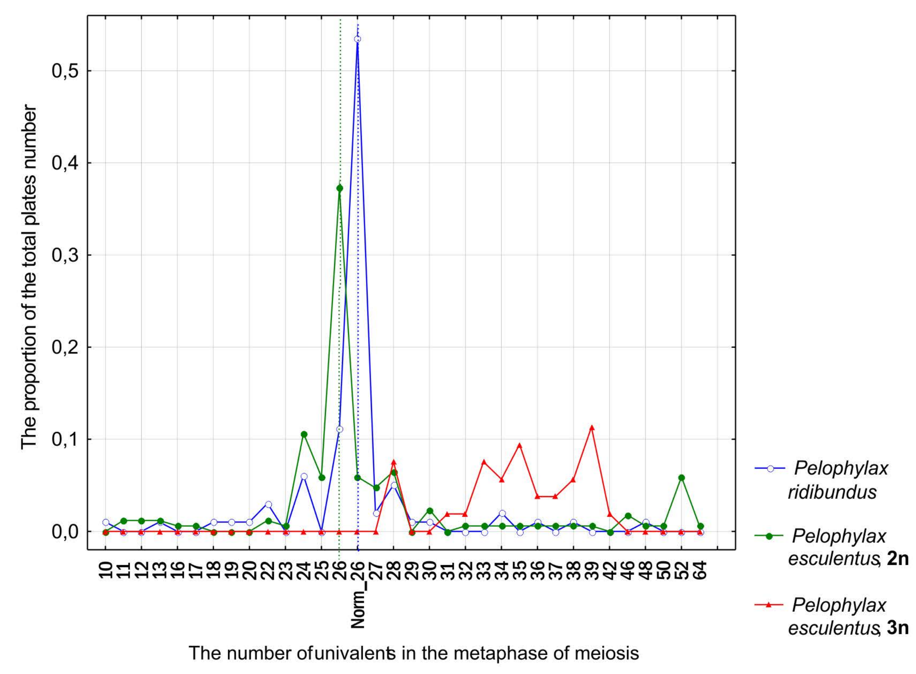

Fig. 4. Frequency of occurrence of meiotic metaphase plates with different number of chromosomes among total amount of mitotic plates analysed from testes of *P. ridibundus*, diploid *P. esculentus* and triploid *P. esculentus*

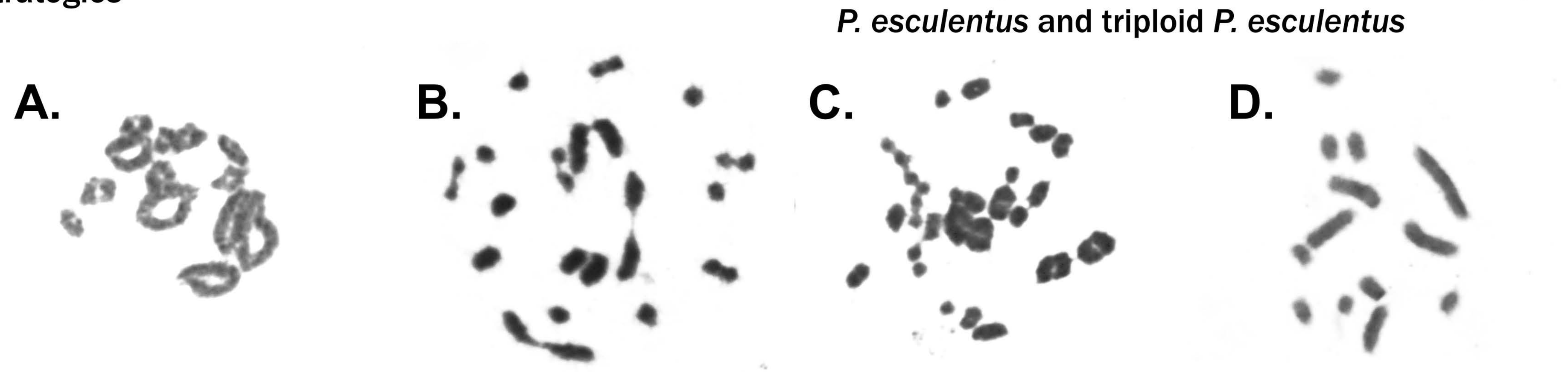

Fig. 5. Spermatocytes I from testes of subadult water frogs: A — normal 2n plate with 13 bivalents, *P. ridibundus*; B — abnormal 2n plate with 8 bivalents and 10 univalents, 2n *P. esculentus*; C — abnormal 3n plate with 11 bivalents, 13 univalents and 1 tetravalent, 3n *P. esculentus*; D — abnormal haploid plate with 13 univalents, 2n *P. esculentus*

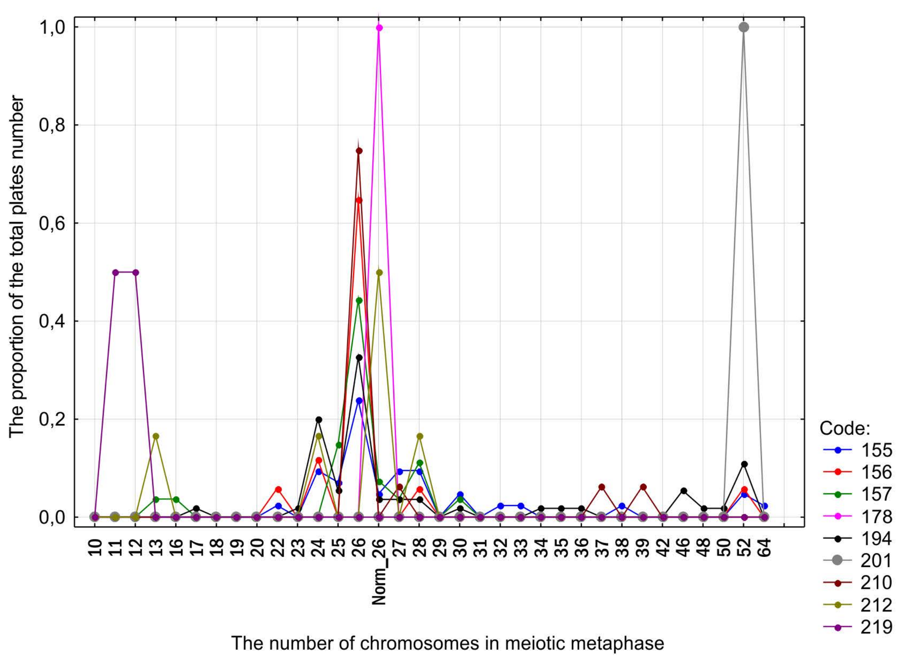

Fig. 6. Distribution of proportion of spermatocytes I with various chromosome number in different diploid individuals *P. esculentus*

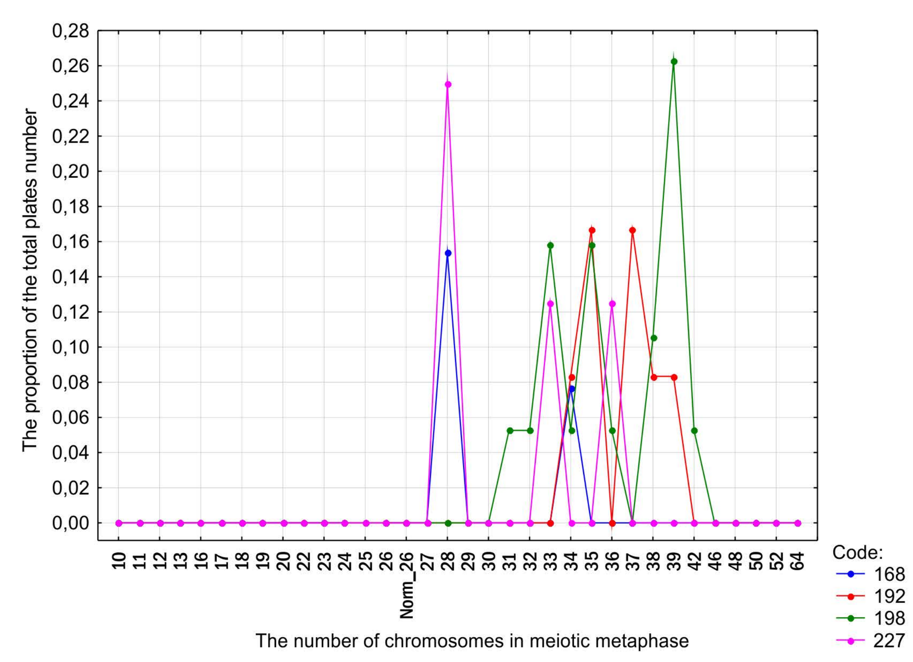

Fig. 7. Distribution of proportion of spermatocytes I with various chromosome number in different triploid individuals *P. esculentus*

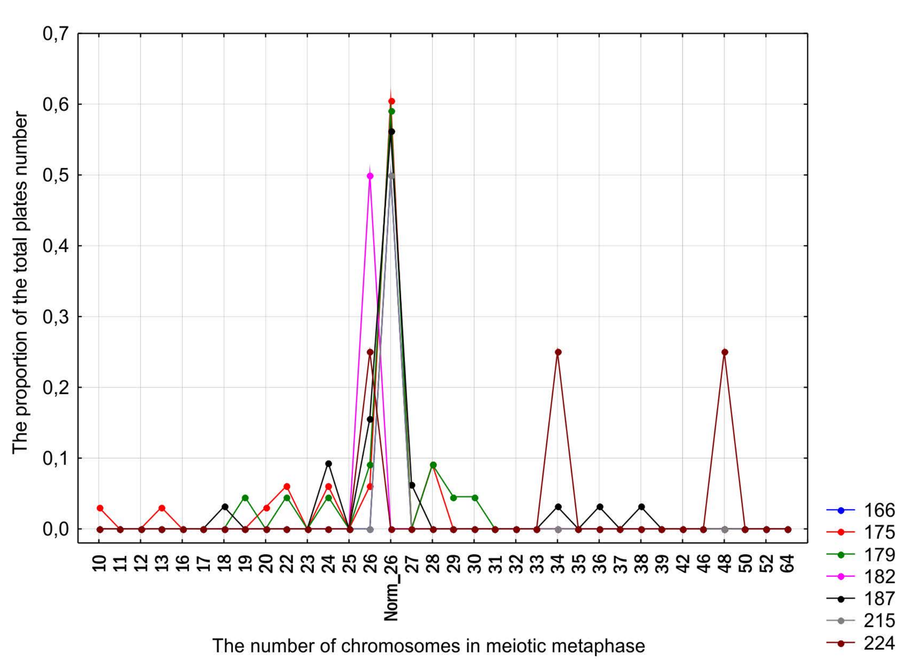

Fig. 8. Distribution of proportion of spermatocytes I with various chromosome number in different individuals *P. ridibundus*

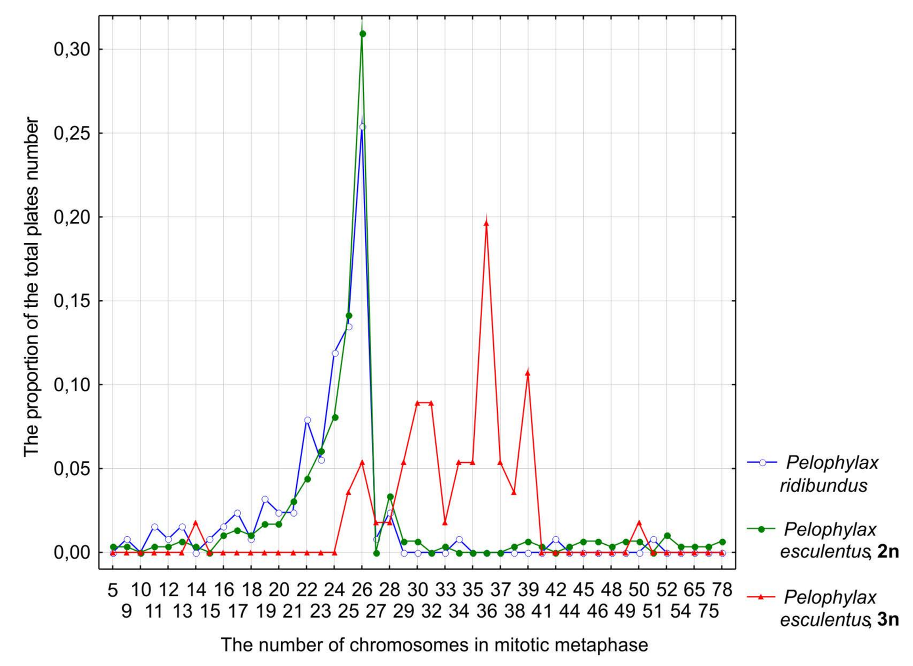

Fig. 9. Frequency of occurrence of the mitotic metaphase plates with a different number of chromosomes among total amounts of mitotic metaphases analysed from testes of *P. ridibundus*, diploid *P. esculentus* and triploid *P. esculentus*

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