PROLIFERATIVE STATUS DETERMINATION OF BENTIC INVERTEBRATES COELOMOCYTES/ HEMOCITES

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Abstract
Flow cytometry was employed to determine the coelomocytes/hemocytes DNA content in five selected marine invertebrates from the Adriatic sea (sea mouse *Aphrodita aculeata*, spiny crab *Maja crispata*, starfish *Echinaster sepositus*, sea urchin *Paracentrotus lividus* and tunicate *Phallusia mammillata*). The cell cycle analyses identified sea mouse coelomocytes as proliferating cells and revealed that spiny crab hemocytes and sea urchin coelomocytes complete their division in the hemolymph and coelom, respectively. The diploid DNA content (2C) in sea mouse was 1.24 pg, spiny crab 7.76 pg, starfish 1.52 pg and sea urchin 1.08 pg
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Marine invertebrates play a very important role in the maintenance of the health of marine ecosystems and serve as bio-indicators. Coelomocytes/hemocytes of benthic invertebrates, as primary immune effectors that respond to stress conditions, injures, host invasion and cytotoxic/genotoxic agents, have been used for the monitoring of environmental conditions [1, 2]. As a free circulating they are easily collected and analyzed by flow cytometry.

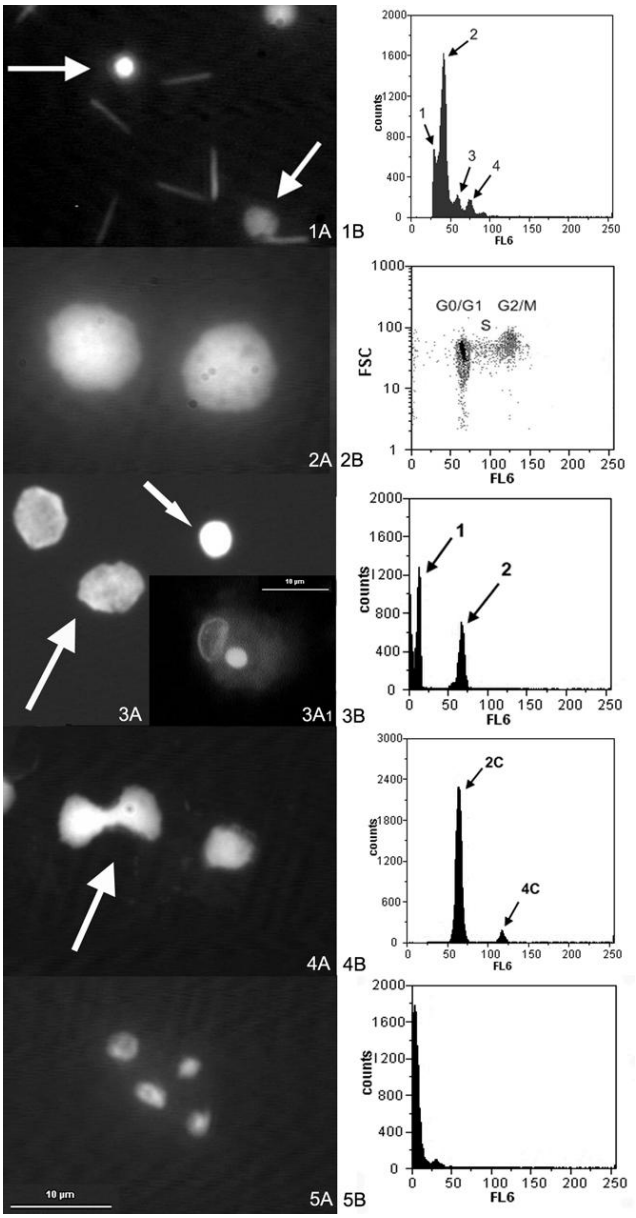

Fig. 1. (1) *Aphrodita aculeata*. (2) *Maja crispata*. (3) *Echinaster sepositus*. (4) *Paracenthrotus lividus*. (5) *Phallusia mammillata*. - (A) DAPI - stained nuclei and cells (3A1). (B) DAPI-fluorescence (FL6) distribution

The DNA content is a marker of cellular maturity since the frequency of cells is G0, S and G2 phases could determine the proliferative status of the investigated cells. When the measurement of DNA content is performed with internal standard, flow cytometry provides information about genome size [3].

Tab. 1. Descriptive statistics of genome size variation within 5 species of marine invertebrates calculated from distribution of DNA values of 10 specimens

Species	DNA content (2C)/pg	Range	CV
Aphrodita aculeata	1.24±0.06	1.15-1.30	4.8
Maja crispata	7.76±0.10	7.63-7.91	1.3
Echinaster sepositus	1.52±0.03	1.46-1.54	2.0
Paracenthrotus lividus	1.08±0.02	1.05-1.12	4.6
Phallusia mammillata	0.11±0.05	0.07-0.14	45

Conclusions

1. Flow cytometry analyses of DAPI-stained nuclei identified the sea mouse *A. aculeate* coelomocytes as proliferating cells.
2. Fluorescence microscopy of DAPI-stained cells identified the presence of phagocytic activity in unchallenged red starfish *E.sepositus*.
3. Evidence of karyokinesis of both spiny crab hemocytes and sea urchin coelomocytes revealed that their division is completed in the hemolymph and coelom, respectively.
4.The diploid DNA content (2C) in sea mouse *A. aculeata*, spiny crab *M. crispata*, red starfish *E. sepositus* and sea urchin *P. lividus* is 1.24, 7.76, 1.52 and 1.08 pg, respectively.
5. DNA content of sea mouse *A. aculeata* and spiny crab *M.crispata* are the first records of their genome size. Furthermore, the DNA content of the sea mouse *A. aculeata* is the first genome size record among the species in the Aphroditidae family.

References
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