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ORIGINAL ARTICLE

SMALL HSPs MOLECULAR WEIGHTS AS NEW INDICATION TO THE HYPOTHESIS OF SEGREGATED STATUS OF THERMOPHILIC RELICT *GMELINOIDES FASCIATUS* AMONG BAIKAL AND PALEARCTIC AMPHIPODS

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Among the great diverse of Baikal fauna of amphipods (more than 350 species and subspecies) *Gmelinoides fasciatus* (Stebbing 1899) takes the special place. It is characterized by a high morphological variability and wide thermal-adaptive possibilities. By common opinion *G. fasciatus* is a thermophilic relict segregated from other Baikal temperature sensitive amphipods' fauna. In present study we tested hypothesis of segregated status of *G. fasciatus* among some Baikal and some Palearctic amphipods using of heat-shock proteins' (HSP) molecular weight as comparative biomarkers. We used heat-shock proteins (HSP) from two families: HSP70 and small HSP (sHSP) immunochemically related to α-crystalline. 15 species of Baikal amphipods from different genera and families and 2 Palearctic species from genus *Gammarus* were tested. It was shown, that molecular weights of HSP70 were the same in all investigated species and corresponds close to 70 kD. In the contrast, the molecular weights of sHSP in *G. fasciatus* was 37 kD and differed from all other species, in which molecular weights of sHSP were 35 kD. In sum, this study showed that sHSPs molecular weights may relate to evolutional differences between the close related species. Additionally, obtained data can be taken as new indication of segregated status of thermophilic relict *G. fasciatus* which linked with its phylogenetic history in Lake Baikal.

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Lake Baikal is a largest center of the fresh-water amphipods diversity in the world. More than 350 species and subspecies are described in the lake (Takhteev, 2000). Baikal amphipods' species have sprung in the lake as a result of endemic speciation. Majority of endemic species are temperature

sensitive and mostly don't spread out of the lake. The exception is *Gmelinoides fasciatus* (Stebbing 1899). It is belonged to the monotypic genus and characterized by a great morphological variation and a high ecological flexibility (Bazikalova, 1945). The genetic polymorphism in the Baikal populations of *G. fasciatus* was also detected (Gomanenko et al, 2005). *G. fasciatus* is thermophilic species inhabits in the shallow waters of the open Baikal, mostly till to the depth of 5 m but it is the most abundant in the shore zone and the well-warmed bays (Florensov, 1977; Timofeyev & Kirichenko, 2004). It can be also found in surrounding water bodies of Baikal region including several thermal spring with temperature close to 29°C (Takhteev, 2009).

In present time the question about origin and age of Baikal amphipods' fauna is under intensive discussion. By common opinion the repetitive ecological crises in Pleistocene caused by climate changes and variations of water levels had strong effect on Lake Baikal fauna. These crises leaded series of great extinctions in ancient Baikal fauna and following for that to new explosions of speciation (Mats & Tcherbakov, 2008 etc.). However, some of thermophilic relict species were survived in the warm refuges (Karabanov et al., 2004). It was hypothesized that *G. fasciatus* is one of these relict species which characterized by a significant phylogenetic segregation (Pleshanov & Tahteev, 2008).

The aim of the present study to test hypothesis of segregated status of *G. fasciatus* among Baikal and some Palearctic amphipods with use of heat-shock proteins' molecular masses as comparative biomarkers.

MATERIALS AND METHODS

Study sites and animals

17 amphipods species from different genera and families, as well as different ecological preferences and habitats were chosen for this study: 15 endemic Baikal species and 2 Palearctic species belongs to genus *Gammarus*. The determination of Baikal species was done by keys of Takhteyev (2000). Animals were collected during 2008 – 2010 years. Characteristics of amphipods taxonomy and sampling points is done in Table 1.

Heat-shock proteins

We used heat-shock proteins (HSP) from two families: HSP70 and small HSP (sHSP) immunochemically related to α–crystalline as comparative biomarkers. Both HSPs are cell chaperones and their main function is a protection of cell proteins from damages caused by a huge variety of stressors as well as a recovery of the native structure and function of the damaged proteins. The chosen HSPs are characterized by the different levels of conservatism. So, the cross-hybridization of DNA and RNA coding HSP70 purified from nematodes, mollusks, fish and mammalian showed a high level of homology (Sanders, 1993). sHSP is a large and heterogenic group, which includes proteins with molecular weight from 12 to 43 kD, which have a high-conservatively region - α-crystalline domain (Arrigo & Muller, 2002).

The HSP content was determined through SDS electrophoresis of equal protein amounts followed by Western blotting according to Bers & Garfin (1985) with modification by Timofeyev et al. (2009). Monoclonal anti-heat shock protein 70 of BRM-22 clone (Sigma) as primary antibody and anti-mouse IgG (Sigma) as secondary antibody were used to identify HSP70 in the samples. Rabbit polyclonal anti-αA/αB-cristallin antibody of the "whole rabbit serum" format (Stressgen) as primary antibody and anti-rabbit IgG (Stressgen) as

secondary antibody were used to identify of sHSP in the samples.

RESULTS

The results of the HSP comparing of the different amphipods species are explained at the fig. 1, 2. The fig. 1 shows that molecular weights of HSP70 are the same in all investigated species and corresponds close to 70 kD. It was also shown the molecular weight of amphipods HSP70 were very close with molecular weight of the purified HSP70 from bovine brain used as positive control.

Other picture presents data for sHSP evaluation (fig.2). The results explained that molecular weights of sHSP were very close and corresponds to 35 kD in the most investigated species. However, *G. fasciatus* significantly differed from the other species. The molecular weight of sHSP of *G. fasciatus* corresponds to 37 kD. The molecular weight of the purified bovine αA/αB- crystalline which was used as positive control also differed and corresponded to 22 kD.

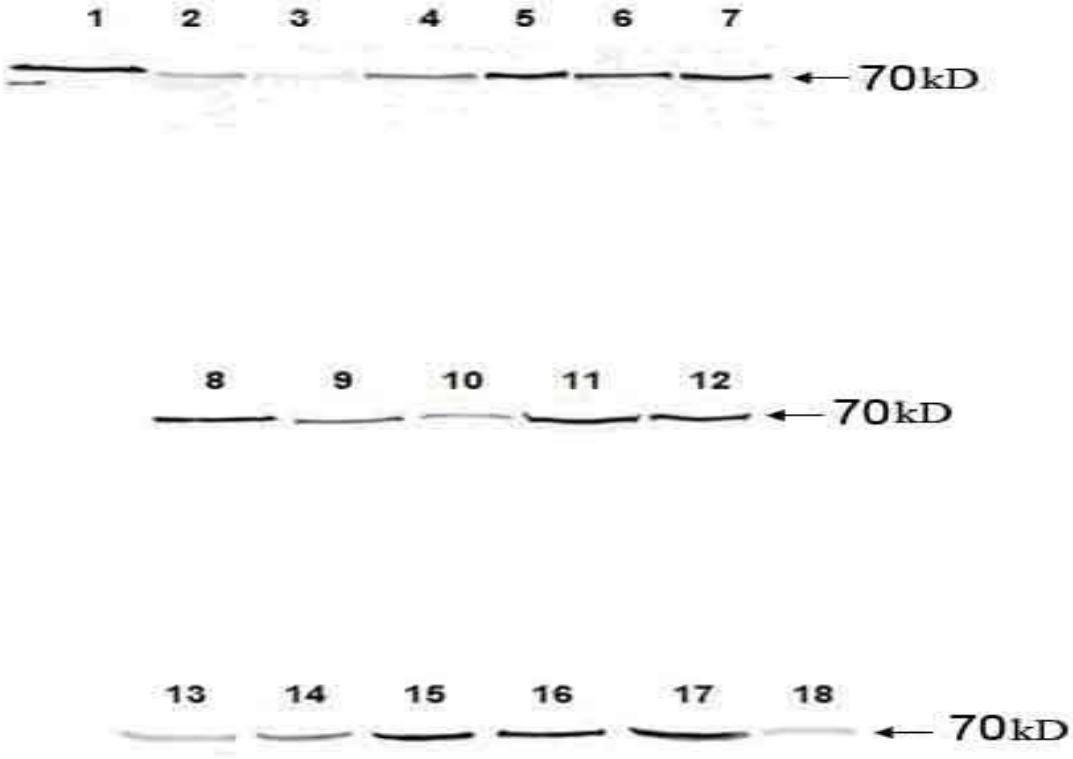

Figure 1 HSP70 in different amphipods' species (results of Western-blotting)
1, 8 – positive control (200 ng); 2 – *Plesiogammarus martinsoni*; 3 – *Parapallasea borowskii*; 4 – *Parapallasea puzyllii nigra*; 5 – *Pallasea grubii*; 6 – *Pallasea cancellus*; 7 – *Pallasea cancelloides*; 9 – *Ommatogammarus flavus*; 10 – *Ommatogammarus albinus*; 11 – *Eulimnogammarus marituji*; 12 – *Eulimnogammarus maakii*; 13 – *Eulimnogammarus verrucosus*; 14 – *Eulimnogammarus vittatus*; 15 – *Eulimnogammarus cyaneus*; 16 – *Gmelinoides fasciatus*; 17 – *Gammarus lacustris*; 18 – *Gammarus pulex*.

Table 1: Sampling points of amphipods

Taxon	Depth habitat (m), type of soil	Sampling point
Family Carinogammaridae		
Gmelinoides fasciatus (Stebbing 1899)	0–5 (to 192) Sa, Si, St, M	Lake Baikal, Bolshie Koty bay
Family Acanthogammaridae		
Acanthogammarus brevispinus Dorogostajsky 1922	3–200 Si, Sa	Lake Baikal, Chivirkuyski bay
Plesiogammarus martinsoni martinsoni Tachteew 1997	40–1240 Si, SiSa	Lake Baikal, South of Maloye More Strait
Family Pallaseidae		
Pallasea cancelloides (Gerstfeldt 1858)	0–178 St, M, Sa	Lake Baikal, Chivirkuyski bay
P. cancellus cancellus (Pallas 1776)	1–52 St, M	Lake Baikal, Chivirkuyski bay
P. grubii grubii (Dybowsky 1874)	1–175 Sa, SiSa, St	Lake Baikal, North of Maloye More Strait (opposite site of Peschanaya Bay)
Parapallasea borowskii borowskii (Dybowsky 1874)	10–1176 Si, SiSa, St	Lake Baikal, Samarinskiy cape
P. puzyllii nigra (Garjajew 1901)	3–250 Si, St	Lake Baikal, Selenga region close to Haraus river
Family Gammaridae		
Eulimnogammarus cyaneus cyaneus (Dybowsky 1874)	0–3.5 St, Si	Lake Baikal, Bolshie Koty bay
E. maackii maackii (Gerstfeldt 1858)	0–40 St	Lake Baikal, Bolshie Koty bay
E. marituji Bazikalova 1945	0–30 St	Lake Baikal, Bolshie Koty bay
E. verrucosus verrucosus (Gerstfeldt 1858)	0–12 St	Lake Baikal, Bolshie Koty bay
E. vittatus (Dybowsky 1874)	0–30 St	Lake Baikal, Bolshie Koty bay
Ommatogammarus albinus (Dybowsky 1874)	47–1641	Lake Baikal, Bolshie Koty bay
O. flavus (Dybowsky 1874)	2 –1313	Lake Baikal, Bolshie Koty bay
Gammarus lacustris Sars 1863	0–22 St, M	Shallow lake of Irkutsk city (app. 60 km from Lake Baikal)
G. pulex (Linnaeus 1758)	0–10 St, Si	Freshwater channel (Kiel, Germany)

Si – silt, **Sa** – sand, **SiSa** – silty sand, **St** – stones, **M** – macrophytes, **Sp** – colonies of sponges.

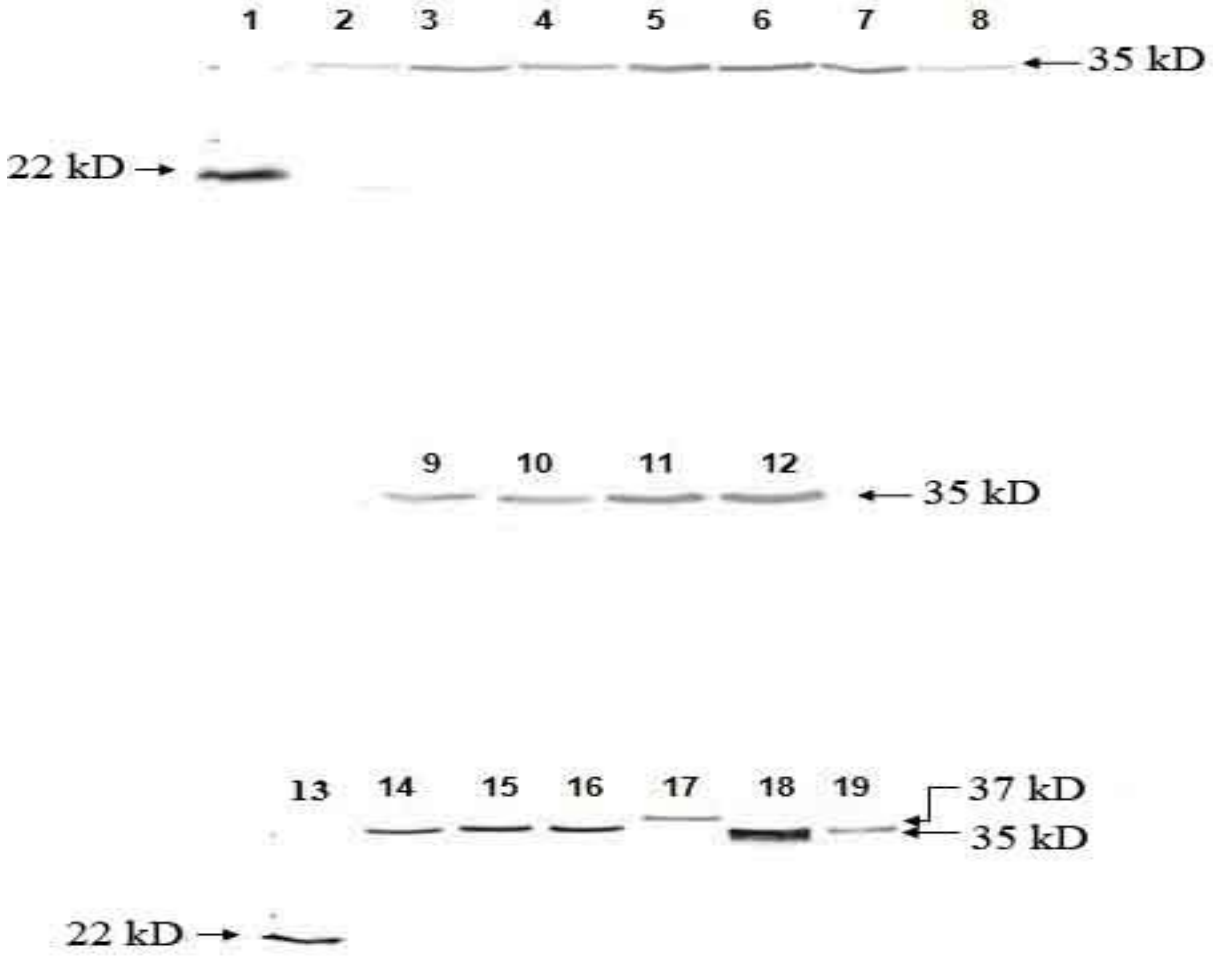

Figure 2 sHSP in different amphipods' species (results of Western-blotting)
1, 13 – positive control, sHSP (150 ng); 2 – *Plesiogammarus martinsoni*; 3 – *Parapallasea borowskii*; 4 – *Parapallasea puzyllii nigra*; 5 – *Pallasea grubii*; 6 – *Pallasea cancellus*; 7 – *Pallasea cancelloides*; 8 – *Acanthogammarus brevispinus*; 9 – *Ommatogammarus flavus*; 10 – *Ommatogammarus albinus*; 11 – *Eulimnogammarus marituji*; 12 – *Eulimnogammarus maakii*; 14 – *Eulimnogammarus verrucosus*; 15 – *Eulimnogammarus vittatus*; 16 – *Eulimnogammarus cyaneus*; **17 –** *Gmelinoides fasciatus***;** 18 – *Gammarus lacustris*; 19 – *Gammarus pulex*.

DISCUSSION

The results obtained confirmed the high conservatism of the proteins from HSP70 family. Molecular weights of HSP70 were very close in the different amphipods' species, genera and families, didn't depend of the ecological characteristics, their life forms or habitat depths. More over the HSP70 molecular weights of crustacean (amphipods) and mammalian (bovine) proteins were also really close.

The coordinately different picture was obtained for sHSP. The results demonstrated a heterogeneity of proteins from this family. Molecular weight of sHSP observed in amphipods was 35-37 kD. This is corresponds to the range 12-43 kD mentioned above. It was also differing from molecular weight

of bovine α-crystalline used as positive control. This fact may link with diversity of sHSP family which combines a lot of proteins which have common α-crystalline domain. The special interest is not only the differences between different phylum (Arthropoda and Chordata) but mainly the observed interspecies differences between relict and contemporary amphipods species. Thus we can suggest that sHSP can relate on phylogenetic differences between Baikal amphipods species more than HSP70.

In sum, this study showed that sHSPs molecular weights may relate to evolutional differences between the close related species. Additionally, obtained data can be taken as new indication of segregated status of thermophilic relict *G. fasciatus* which linked with its phylogenetic history in Lake Baikal.

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