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# Allozyme Variation and Genetic Relationships within the *Phrynocephalus guttatus* Species Group (Sauria: Agamidae) in the Former USSR.

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***Abstract.*** -An electrophoretic analysis of several populations of *Phrynocephalus guttatus* s. lato. shows that there are two groups with a remarkable level of genetic differentiation. There is an eastern Palearctic *P. versicolor* from southern Mongolia, and a western Palearctic *P. guttatus* s. str. which includes: *g. guttatus, g. kushackewitschii g. alpherakii, g. salenskyi, g. moltschanovii, guttatus ssp.* from northern Turkmenia and *versicolor hispida* from Djungar Gate. There are no objective criteria for subspecific separation by biochemical genetic markers.

*Key words:* Reptilia, Sauria, Agamidae, *Phrynocephalus*, electrophoresis, systematics.

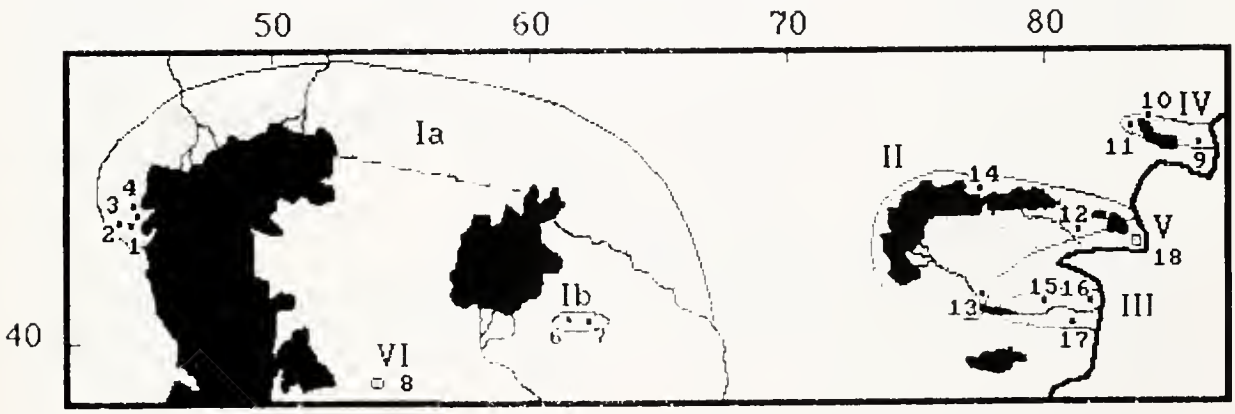


FIG. 1. Distribution of the Phrynocephalus guttatus species group in the former USSR. Ia- P. g. guttatus; Ib- P. g. moltschanovi; II- P. g. kushackewitschii; III- P. g. alpherakii; IV- P. g. salenskyi; V- P. versicolor hispida; VI-P. guttatus ssp. The numbering of the populations is as given in Table 1.

## Introduction

The agamid genus *Phrynocephalus* includes some polytypic species groups. One of the most complicated species complexes is *Phrynocephalus guttatus* s. lato. Representatives of this species group are widely distributed in Middle and Central Asia from the northern Caucasus to China. The systematics of this species group is highly controversial and needs revision.

There are some alternative viewpoints on the status and systematic relationships of its representatives. The classical viewpoint of Terentjev and Chernov (1949) recognized only two species: *P. versicolor* and *P. guttatus (P. g. guttatus* and *P. g. kushackewitschii).* A new concept was developed during the last decade by Semenov and Shenbrot (Semenov and Shenbrot, 1982; Shenbrot and Semenov, 1987; Semenov et. al., 1987). According to this concept, the *guttatus*-group consists of four species: *P. guttatus* (Gmel), P. *moltschanovi* Nik., *P. melanurus* Eichw. (=*P. salenskyi* Bedr.) and *P. versicolor* Str. The last form includes the nominal subspecies (China: Alashan to Djungaria), *P. v. kulagini* (Tuva, Russia; western Mongolia) and the western Palearctic subspecies, *P. v. paraskii* Semenov, Brushko, Kubykin et Shenbrot. Golubev (1989) lowered the status of *"salenskyi"* to subspecific level, united *P. v. paraskii* with *P. guttatus alpherakii* Bedr. and included *"moltschanovi"* only as a color variation of *P. g. guttatus.*

TABLE 1. Localities, sample sizes and taxa of *Phrynocephalus guttatus* S. lato. populations collected and investigated in this study.

| N | Taxa | Locality | No. |
|---|---|---|---|
| 1 | *P. g. guttatus* | NORTHERN TRANSCAUCASUS REGION | |
| | | Daghestan: Tersky Sands near Chervlenny Buruny | 2 |
| | | Russia: Stavropol Dist., Tersky Sands: Roshchino | 3 |
| | | Chencheno-Ingushety: near Starogladkovsky | 3 |
| | | N. Daghestan: sands on the right bank of Kuma River | 1 |
| 2 | *P. g. moltschanovi* | N. Kysylkum in Karakalpakia: Beltau Mount. | 4 |
| | | N. Kysylkum in Karakalpakia: Kostruba Well | 5 |
| 3 | *P. guttatus ssp.* | N.Turkmenia: Kazakhlyshor near Kumsebshen Well | 3 |
| 4 | *P. g. salenskyi* | E. KAZAKHSTAN: Zaissan Depression: | |
| | | Left bank fo Black Irtysh near Karatal | 22 |
| | | Irtysh Sands near Chingildy | 18 |
| | | Left bank of Bukhtarma Reservior: Kuludjunsky Sands | 18 |
| 5 | *P. g. kushackewitschii* | E. KAZAKHSTAN: Taldy-Kurghan Dist. (Alakol Basin): | |
| | | Near Andreevka (left bank of Chyndjaly River | 23 |
| 6 | *P. g. kushackewitschii* | E. KAZAKHSTAN: Taldy Kurghan Dist. (Balkhash Basin): | |
| | | NW bank of Kapchagay Reservior | 10 |
| | | SW of Balkhash: Ortadyressin | 2 |
| 7 | *P. g. alpherakii* | E. KAZAKHSTAN: Taldy-Kurghan Dist. (right bank of Illi ); | |
| | *(+P. v. paraskii* | Panfilov Region: 25 km. from Aidarly | 2 |
| | | Kerbulak Region: Ayakkalkan | 7 |
| | | Alma-Ata Dist.: near Chundja (left bank of Illi) | 2 |
| 8 | *P. versicolor hispida* | E. Kazakhstan: Djungar Gate | 7 |
| 9 | *P. v versicolor* | Mongolia: South Gobi Aimag: Dalanzadagad | 5 |
| 10 | *P. strauchi* | Fergan Valley: left bank of Kajrakkum Reservior near Kyly | 20 |
| 11 | *P. helioscopus saidalievi* | S. part of Fergan Valley near Kim | 7 |

A high level of morphological variation on the one hand, and caryological conservatism on the other, doesn't allow one to decide problems of systematic status and specific identity of representatives of *P. guttatus* s. lato. Therefore, in order to decide controversial systematic problems of this agamid group, we used biochemical genetic markers.

## Methods

Electrophoretic analysis was carried out on geographic forms of *P. guttatus* s. lato from different geographic regions (Fig. 1, Table 1). The geographic form from northern Turkmenia was excluded from *P. g. guttatus* on the basis of the red spots on the arm pits, a very rare characteristic in *P. guttatus*. We also studied two well differentiated species, *P. strauchi* Nik. and *P. helioscopus saidalievi* Sattorov, both from the Fergan Valley, as an external control for genetic differentiation.

Each adult specimen was processed in the laboratory for blood and muscle samples and immediately studied by standard vertical acrylamide electrophoresis. Homogenates obtained from muscle, crushed in distilled water with 5 per cent sucrose, were processed for the following enzymes and proteins (Table 2).

Isozymes were numbered in order of decreasing mobility from the most anodal one. Allozymes were designated numerically according to their mobility, relative to the most frequent allele (100), faster mobility (>100), slower mobility (<100). The genetic divergence between populations and divergence time were estimated with indices of standard genetic distances by formulas proposed by Nei (1975).

TABLE 2. Enzymes studied and electrophoretic conditions employed.

| Enzyme or protein | Locus abrev. | EC no. | Tissue | Buffer |
|---|---|---|---|---|
| Aspartate aminotransferase | s-Aat | 2.6.1.1 | Muscle | TEB |
| Glycerol-3-phospate dehydrogenase | G-3-pdh | 1.1.1.18 | Muscle | TEB |
| Isocitrate dehydrogenase | s-Idh | 1.1.1.42 | Muscle | TEB |
| Lactate dehydrogenase | Ldh-A | 1.1.1.27 | Muscle | TEB |
| Lactate dehydrogenase | Ldh-B | 1.1.1.27 | Muscle | TEB |
| Malate dehydrogenase | s-Mdh | 1.1.1.37 | Muscle | TEB |
| Malic enzyme | s-Me | 1.1.1.40 | Muscle | TEB |
| Superoxide dismutase | s-Spd | 1.15.1.1 | Muscle | TEB |
| 6-phosphogluconate dehydrogenase | 6-pgdh | 1.1.1.44 | Muscle | TEB |
| Phosphoglucomutase | Pgm | 2.7.5.1 | Muscle | TG |
| Esterase | Es-D | 3.1.1.1 | Muscle | TEB |
| Esterase | Es-2 | 3.1.1.1 | Muscle | TG |
| Esterase | Es-3 | 3.1.1.1 | Hemolizate | TG |
| Hemoglogin | Hb | - | Hemolizate | TG |
| Albumin | Alb | - | Muscle | TG |
| Structural muscle proteins | Pt-1. 2. 3 | - | Muscle | TG |

Note: TEB- Tris-EDTA-$NA_2$ boric acid Ph 8.5 (Peacock et al., 1965). TG- Tris-glycin, disc-electrophoresis (Davis, 1964)

## Results

*Allozyme variation.*—Four of 18 loci analyzed (Es-3, Pt-2, Pt-3, IdhS) were monomorphic and fixed for the same allele in all populations and species considered. Fourteen loci were polymorphic within or between population and their allelic frequencies are given in Table 3. Expected genotypes distributions were in equilibrium according to Hardy-Weinberg formula in the investigated populations at all loci observed. Exceptions were obtained only in *"salenskyi"* and *"kushackewitschii"* at the Ldh-B locus (Table 4). In our opinion, absence of heterozygote genotypes can be explained by introgression of Ldh-B 90 from *"salenskyi"* to *"kushackewitschii"* or vice versa.

Levels of genetic variation are given in Table 3 (Mean proportion of heterozygosity observed (H obs.) and expected (H exp.)}. H obs. ranged from 0 in *"salenskyi"* to 0.05 in "kushackewitschii" with a mean of 0.04. This meaning of heterozygosity is near the level usual for Reptilia (Nevo, 1984).

*Genetic divergence.*—Only two loci (Me-S, Mdh-S) display fixation or predominance of alternative alleles between *P. versicolor* (Mongolia) and the *P. guttatus* group. All representatives of Western palearctic *P. guttatus* have common gene pools of the loci considered. *Phrynocephalus. v. hispida* is an exception and has alternative allelic fixation at Es-2 (Table 3). Genetic distances among *P. guttatus* forms are rather low and range from 0 to 0.05 i.e. on interspecific level of differentiation.

There are four loci which display alternative fixation between *P. strauchi* and *P. guttatus*. It shows a clear intraspecific level of genetic divergence. The largest genetic distance is between *P. h. saidalievi* and *P. guttatus* species group (D=0.832). Differences between these species include 8 loci which display alternative allelic fixation (Pgm, Mdh-S; Aat-S, Me-S, Alb; Pt. 1; Ldh-B, Es-2).

From the allelic frequencies at 18 loci tested, we calculated Nei's genetic distance and constructed a matrix of genetic distances (Table. 5). A UPGMA phenogram was calculated on the basis of this matrix. This reflects the relationships

TABLE 3. Allelic frequencies.

| Locus | Allele | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| s-Aat | 90 | | | | | | | | | | | 0.97 |
| | 95 | | | | | 0.12 | | | | | | 0.03 |
| | 100 | 1.00 | 1.00 | 1.00 | 0.98 | 0.88 | 1.00 | 1.00 | 1.00 | 1.00 | 0.80 | |
| | 105 | | | | 0.02 | | | | | | 0.20 | |
| G-3-pdh | 95 | | 0.25 | | | | | | | | | |
| | 100 | 1.00 | 0.75 | | | | | | | | | |
| Ldh-A | 90 | | 0.25 | | 0.08 | 0.92 | 0.81 | 0.39 | | 0.67 | | |
| | 100 | 1.00 | 0.75 | 1.00 | 0.92 | 0.08 | 0.19 | 0.61 | 1.00 | | 1.00 | 1.00 |
| Ldh-B | 90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | | |
| | 100 | | | | | | 0.03 | | | | 1.00 | 1.00 |
| s-Mdh | -100 | | | | | | | | | 0.93 | | |
| | 80 | | | | 0.02 | | | | | 0.17 | | |
| | 90 | | | | | | | | | | | 1.00 |
| | 100 | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | | 1.00 | |
| s-Me | -100 | | | | | | | | | 0.50 | | |
| | 90 | | | | | | | | | | 1.00 | |
| | 91 | | | | | | | | | | | 1.00 |
| | 98 | | | | | | | | | 0.40 | | |
| | 100 | 1.00 | 1.00 | 1.00 | 0.20 | 0.83 | 1.00 | 1.00 | 1.00 | 0.10 | | |
| | 102 | | | | 0.53 | 0.13 | | | | | | |
| | 105 | | | | 0.14 | 0.04 | | | | | | |
| | 108 | | | | 0.11 | | | | | | | |
| Pgm | 95 | | | | | | | | - | 0.17 | | |
| | 100 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | | 0.83 | 1.00 | |
| | 102 | | | | 0.03 | | | | | | | 1.00 |
| | 105 | | | | | | | | | | | |
| 6-pgdh | 80 | | | | 0.03 | 0.04 | | | | 0.08 | | |
| | 85 | | | | | | | 0.08 | | 0.32 | | |
| | 88 | | | | 0.01 | | | 0.04 | | | | |
| | 90 | 1.00 | 1.00 | 1.00 | 0.96 | 0.96 | 1.00 | 0.88 | 1.00 | 0.60 | | |
| | 98 | | | | | | | | | | | 0.03 |
| | 100 | | | | | | | | | | | 0.94 |
| | 103 | | | | | | | | | | | 0.03 |
| | 108 | | | | | | | | | | 0.05 | |
| | 110 | | | | | | | | | | 0.87 | |
| | 112 | | | | | | | | | | 0.08 | |
| s-Sod | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.91 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 110 | | | | | | 0.09 | | | | | |
| Es-D | 95 | | | | 0.10 | | | | | | | |
| | 98 | | | | | | | 0.23 | | | | |
| | 100 | 0.95 | 1.00 | 1.00 | 0.86 | 1.00 | 1.00 | 0.77 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 105 | 0.05 | | | 0.04 | | | | | | | |
| Es-2 | 94 | | | | | | | | | 0.17 | | |
| | 96 | | 0.12 | 0.25 | 0.50 | 0.04 | 0.05 | 0.55 | | | 0.57 | |
| | 98 | 1.00 | 0.88 | 0.75 | 0.50 | 0.92 | 0.95 | 0.35 | | 0.50 | 0.37 | |
| | 100 | | | | | 0.04 | | | | 0.33 | 0.07 | |
| | 103 | | | | | | | | | | | 1.00 |
| | 105 | | | | | | | 0.05 | 0.21 | | | |
| | 110 | | | | | | | 0.05 | 0.70 | | | |
| | 115 | | | | | | | | 0.09 | | | |
| Hb | 99 | | | | | | | | | | | 1.00 |
| | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | |
| | 102 | | | | | | | | | | 1.00 | |
| Alb | 98 | | | | | | | | | | | 1.00 |
| | 100 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | |
| Pt-1 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | |
| | b | | | | | | | | | | | 1.00 |
| H obs. % | | 0.6 | 3.0 | 2.2 | 6.3 | 4.9 | 3.4 | 6.9 | 4.0 | 6.6 | 5.6 | 1.0 |
| H exp, % | | 0.6 | 3.2 | 2.1 | 8.0 | 5.0 | 4.3 | 7.8 | 3.8 | 13.7 | 5.8 | 1.0 |

Under the electrophoretic condition used, the following loci are monomorphic: s-Idh, Es-3, Pt-2, Pt-3. See table 1 for population numbers.

between the *P. guttatus* s. lato representatives and the other two species that we studied (Fig. 2).

## Discussion

Two distinctive gene pools, differentiated from one another only by two diagnostic loci, were found between representatives of *P. guttatus* s. lato and *P. versicolor* (southern Mongolia). The genetic differentiation corresponds to the division of eastern Palearctic *(P. versicolor* from southern Mongolia) and Western

TABLE 4. Distribution of genotypes at the Ldh-A locus in populations of different geographiac forms of toad agamas of the *Phrynocephalus guttatus* group.

| Form | Locality | | Genotypes distribution | | | |
|---|---|---|---|---|---|---|
| *salensky* | Zaissan | O | 42 | 0 | 6 | |
| | | E | 36.8 | 10.5 | 0.75 | $x^2$ =13.44** d. f. =1 |
| *kushackevitschi* | Andreevka | O | 2 | 0 | 10 | |
| | | E | 0.31 | 3.2 | 8.5 | $x^2$ =4.05* d. f. =1 |
| *kushackevitschi* | Kapchagay | O | 1 | | | |
| | | E | 0.6 | | | |
| *moltschanovi* | Beltau | O | 7 | | | |
| | | E | 6.75 | | | |

Note: O- observed distribution; E- expected distribution; *p < 0.05; **p < 0.001

TABLE 5. Matrix of genetic distances (D, Nei, 1975) among the taxa *P. guttatus* s. lato, *P. strauchi*, and *P. helioscopus saidalievi*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | x | 0.004 | 0.003 | 0.043 | 0.053 | 0.038 | 0.033 | 0.046 | 0.151 | 0.257 | 0.809 |
| 2 | | x | 0.002 | 0.041 | 0.028 | 0.012 | 0.016 | 0.042 | 0.128 | 0.281 | 0.820 |
| 3 | | | x | 0.032 | 0.055 | 0.041 | 0.019 | 0.035 | 0.146 | 0.269 | 0.795 |
| 4 | | | | x | 0.078 | 0.078 | 0.040 | 0.049 | 0.132 | 0.234 | 0.878 |
| 5 | | | | | x | 0.003 | 0.043 | 0.099 | 0.117 | 0.344 | 0.920 |
| 6 | | | | | | x | 0.032 | 0.084 | 0.120 | 0.326 | 0.896 |
| 7 | | | | | | | x | 0.043 | 0.133 | 0.280 | 0.845 |
| 8 | | | | | | | | x | 0.165 | 0.303 | 0.784 |
| 9 | | | | | | | | | x | 0.388 | 0.749 |
| 10 | | | | | | | | | | x | 0.659 |
| 11 | | | | | | | | | | | x |

Palearctic forms *(P. guttatus)* which diverged around 500,000 years ago (Late Pleistocene).

*P. guttatus* consists of conspecific forms, diverse morphologically, but conservative on the molecular level. In this species, the more differentiated form is *P. v. hispida.* This is supported by the fixation of Es-2 (94) which is absolutely absent in *P. v. versicolor.*

The level of genetic differentiation of *P. guttatus* s. lato from *P. strauchi* and *P. helioscopus saidalievi* is higher and corresponds to good species which diverged about 1,500,000-2,000,000 years ago, i.e. in Middle or Early Pleistocene.

On the basis of the data we obtained, our main conclusion is that *P. guttatus* s. lato. consists of two groups with a remarkable level of genetic differentiation. There is an eastern Palearctic *P. versicolor* from southern Mongolia, and a western Palearctic *P. guttatus* s. str.* which includes: *g. guttatus, g. kushackewitschii g. alpherakii, g. salenskyi, g. moltschanovii, guttatus ssp.* from northern Turkmenia and *versicolor hispida* from Djungar Gate. There are no objective criteria for subspecific separation by biochemical genetic markers.

*This abbreviation, which as generally known means "sensu stricto", was erroneously deciphered as "s. Strauch" (Mezhzherin and Golubev, 1992). This somewhat distorted the intended meaning.

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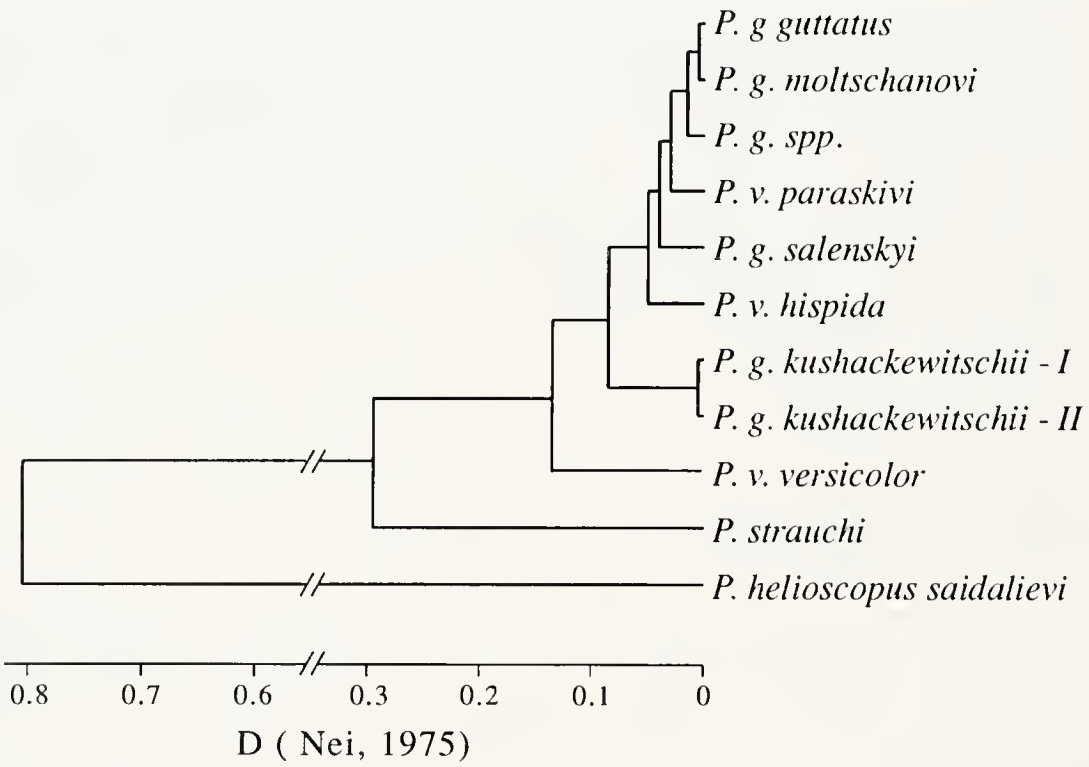


FIG. 2. UPGMA Phenogram of relationships among *Phrynocephalus guttatus* s. lato.

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