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GENERAL BIOLOGY

Phylogenetic Position of the Gansu Mole *Scapanulus oweni* Thomas, 1912 and the Relationships Between Strictly Fossorial Tribes of the Family Talpidae[1]

A. A. Bannikova[a], E. D. Zemlemerova[a], V. S. Lebedev[a], D. Yu. Aleksandrov[b], Yun Fang[c], and B. I. Sheftel[b]

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Abstract—The results of the first molecular study focused on the phylogenetic position of the Gansu mole, *Scapanulus oweni* are presented. The analysis based on sequences of the mitochondrial *cytb* gene and five nuclear genes supports the monophyly of the Scalopini tribe including *S. oweni* and shows that two highly fossorial talpid tribes, Talpini and Scalopini, are not immediate sister taxa. These results highlight the role of morphological parallelism as a potential source of conflict between molecular and morphology-based phylogenies in Talpidae.

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While essential progress was achieved during the last years in the talpid molecular phylogenetics, many studies focus mainly on the relationships of species and genera within tribes [1–4]. The order of divergence and relationships at the tribal level remains an unsolved problem in the phylogenetic history of Talpidae.

Two groups of moles—the tribes Talpini (*Euroscaptor, Mogera, Talpa, Parascaptor, Scaptochirus*) and Scalopini (*Scalopus, Scapanus, Parascalops, Scapanulus*) show highly specialized adaptations to subterranean life. The former tribe is restricted in its distribution to Eurasia while the second is represented now mainly by North American taxa with monotypic *Scapanulus* from Central China being an exception. Whereas the monophyly of Talpini has been confirmed by mitochondrial and nuclear DNA analyses [3], the monophyly of Scalopinae has not been proved with certainty due to the absence of molecular data for *Scapanulus oweni*. Given general chromosomal stability among talpids [5], the recently described karyotype of *S. oweni* (2n = 34, NFa (autosomal fundamental number) = 64) does not provide information which could help to resolve phylogenetic relationships of this species.

Concerning morphology, the available evidence is controversial in many respects. Myological data as well as the parsimony analysis of the skull characters of the present-day genera of Talpidae do not support the monophyly of Scalopini [7]. In the latter case Scalopini is divided into two clades, which are placed in polytomy with the majority of other genera. The monophyly of the whole tribe including *S. oweni* was recovered only in the combined parsimony analysis of cranial and postcranial characters. Here, the Gansu mole appeared as the sister taxon to the North-American *Parascalops breweri* [8]. According to the above analysis [8] Scalopini and Talpini (along with semifossorial *Scaptonyx fusicaudus*) are sister groups, which may be supportive of a single origin of highly specialised subterranean adaptations in evolution of Talpidae.

Thus, according to the results of recent morphological studies [8] *S. oweni* is the only contemporary Asian representative of the mainly American tribe Scalopini. The range of this species is restricted to a limited area, which includes the south of Gansu, Shaanxi, northern Sichuan, and easternmost Qinghai. The pattern of geographical distribution, controversial results of morphological studies and a lack of genetic data for this species provide the basis to question the monophyly of the Scalopini tribe.

The aim of the study was to determine the phylogenetic position of *S. oweni* using molecular methods, to test the monophyly of Scalopini and to evaluate the hypothesis on close relationship between the two

[1] The article was translated by the authors.

[a] *Moscow State University, Moscow, 119992 Russia*
[b] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 117071 Russia*
[c] *Institute of Zoology, Chinese's Academy of Science, Beijing, People's Republic of China*
e-mail: zemlemerovalena@ya.ru, hylomys@mail.ru

Table 1. The list of species and GenBank accession numbers of specimens used in the study

Species	Genes					
	cytb	*RAG1*	*BRCA1*	*BRCA2*	*ApoB*	*A2ab*
T. europaea	**KF801564**	**KP717261**	**KP717157**	**KP717098**	**KP717207**	**KP995402***
T. caucasica	**KP717353**	**KP717298**	**KP717187**	**KP717127**	**KP717240**	**KP995401***
T. occidentalis	**KP717326**					
T. altaica	**FN640579**					
M. wogura	HG737874					
M. robusta	**KC481328**	**KC481361**	**KC481351**	**KP717144**	**KP717255**	**KP995403***
M. latouchei	**KP717378**	**KP995374***	**KP995394***	**KP995387***	**KP995380***	**KP995404***
M. imaizumii	AB638496					
M. tokudae	AB638532					
E. parvidens	**KC481344**					
	KC481339	**KC481366**	**KC481355**	**KP717147**	**KP717257**	**KP995408***
Euroscaptor sp.	**KC481347**	**KC481369**	**KC481357**	**KP717146**	**KP717258**	**KP995409***
E. longirostris	HG737870	HG737914	HG737899			
	HG737871					
E. subanura	**KP995370***	**KP995375***	**KP995395***	**KP995388***	**KP995381***	**KP995410***
E. mizura	AB823104	AB823176	HG737898		DQ630168	
	AB823105					
Scaptochirus moschatus	AB306502	HG737927	HG737911			
	HG737883					
Parascaptor leucura	HG737877	HG737921	HG737905			
	HG737880					
Parascalops breweri	AB076808	**KP717318**	**KP717204**	**KP717149**	**KP717149**	**KP995406***
Scapanulus oweni	**KP995371***	**KP995376***	**KP995397***	**KP995389***	**KP995382***	**KP995411***
	KP995372*	**KP995377***	**KP995398***	**KP995390***		
	KP995373*		**KP995396***			
Scapanus townsendii	AB076820					**KP995412***
Scapanus orarius	AB076817					**KP995413***
Scapanus latimanus	AB076814					
Scalopus aquaticus	AB076809		AF284007			
Condylura cristata	AB076810	**KP717319**	**KP717205**	**KP717150**	**KP717260**	**KP995407***
	AB076811					
Scaptonyx fusicaudus	AB106229	AB106241				
Urotrichus talpoides	AB076832	AB106245				
	AB099483					
Dymecodon pilirostris	AB076830					
	AB076831					
Galemys pyrenaicus	AY833419	AY833415	AY121757			AY121767
Desmana moschata	AB076836	**KP717317**	**KP717203**	**KP717148**		**KP995405***
Uropsilus sp.	DQ630424		DQ630278		DQ630199	AY121768
Uropsilus gracilis	KF778209	KF778311				
Neurotrichus gibbsii	AB076827					
	AB076826					
Crocidura fuliginosa	AB175079					
Crocidura olivieri		**KP995378***	DQ630214		**KP995383***	
Sorex araneus	JN984089	KC113264	**HM036123**	**KP995391***	**KP995384***	XM004609236
Neomys fodiens			**KP995399***	**KP995392***	**KP995385***	
Anourosorex squamipes			**KP995400***	**KP995393***	**KP995386***	
Erinaceus europaeus		**KP995379***	AF284008	JN414309	JN414024	JN413836

Our sequences submitted to GenBank earlier are given in bold,
*—the sequences obtained in this study

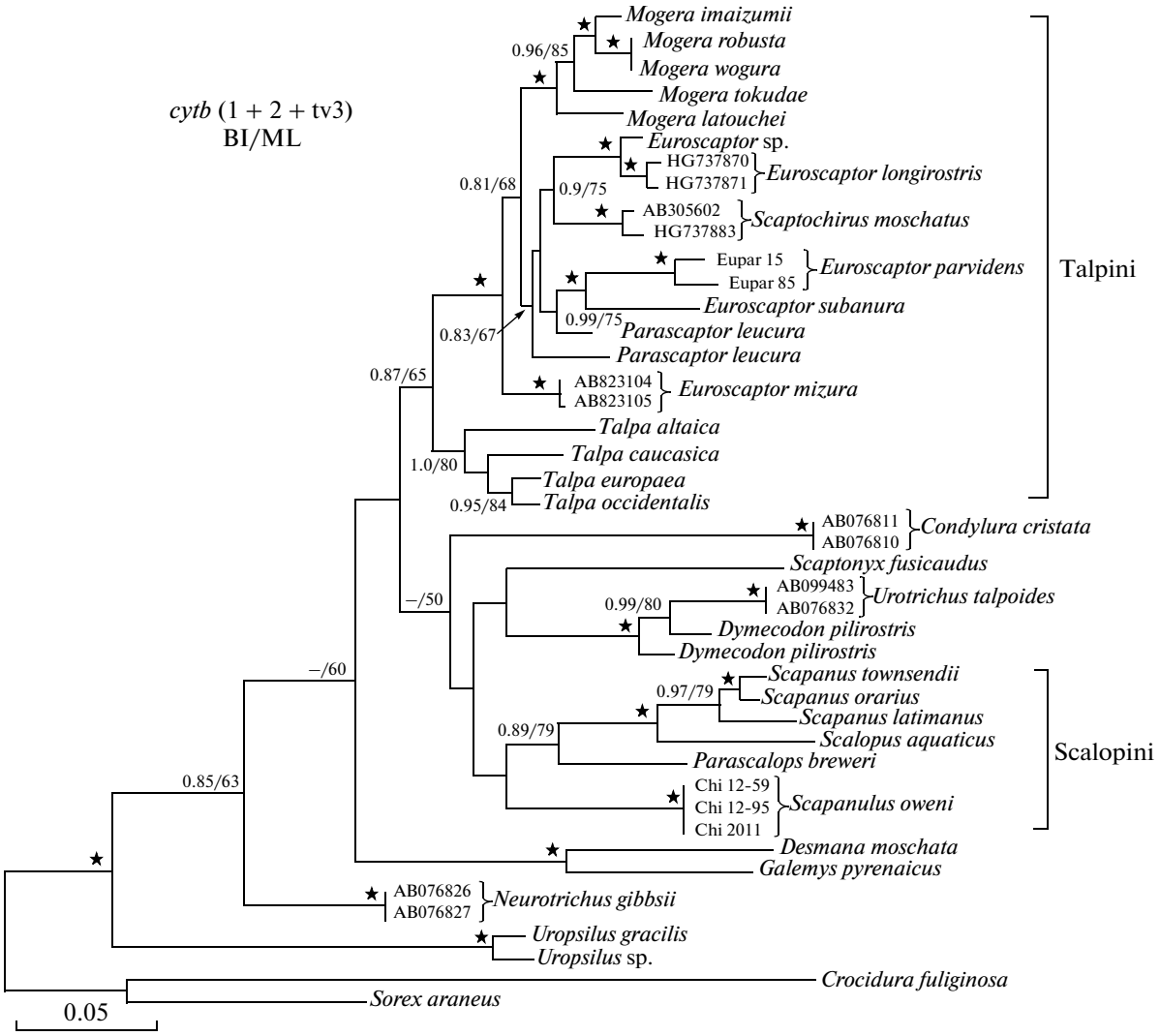

Fig. 1. The mitochondrial ML phylogeny of Talpidae, based on all substitutions at the 1st and 2nd codon positions and transversions at the 3rd position of the complete *cytb* sequence (1140 bp). Values above the branches correspond to Bayesian posterior probabilities in BI and bootstrap support (1000 pseudoreplicates) in ML (BI/ML). The asterisks denote the nodes that are highly supported in both analyses.

branches of strictly fossorial moles. Our study presents the first mitochondrial and nuclear data for *S. oweni*.

Three specimens of Gansu mole were collected in 2011–2012 years during the survey of the fauna of the surroundings of Lianhuashan Natural Reserve in the South Gansu, China. Among them, one specimen was represented by partly decomposed remains of head, skin and tail, apparently it had fallen prey to a terrestrial predator. The other two specimens were found dead on a road and were just slightly damaged.

To clarify the systematic position of the Gansu mole we sequenced fragments of five nuclear genes (*BRCA1* exon 11, *BRCA2, RAG1, ApoB* and *A2ab*) and the complete mitochondrial cytochrome *b* gene (*cytb*) in 33 specimens of 13 taplid species. Besides, 40 sequences of *cytb* of talpides and 12 sequences of other Lipothyphla, submitted earlier in GenBank by us, as well as other 57 sequences from GenBank [3, 4, 9–11] were used in phylogenetic analyses (table).

We reconstructed the mitochondrial tree based on all substitutions at the 1st and 2nd codon positions and transversions at the 3rd positions of the complete *cytb* sequence (1140 bp) as well as the nuclear combined tree based on the concatenation of five nuclear genes (5052 bp). In addition, the phylogenetic analysis of the combined nuclear-mitochondrial data set (5812 bp) was performed using all nuclear data and conservative substitutions of *cytb* (transversions at the 1st codon positions and all substitutions at the 2nd codon positions). Phylogenetic trees were generated by maximum likelihood (ML) and Bayesian inference (BI). In the phylogenetic analysis of nuclear genes two inde-

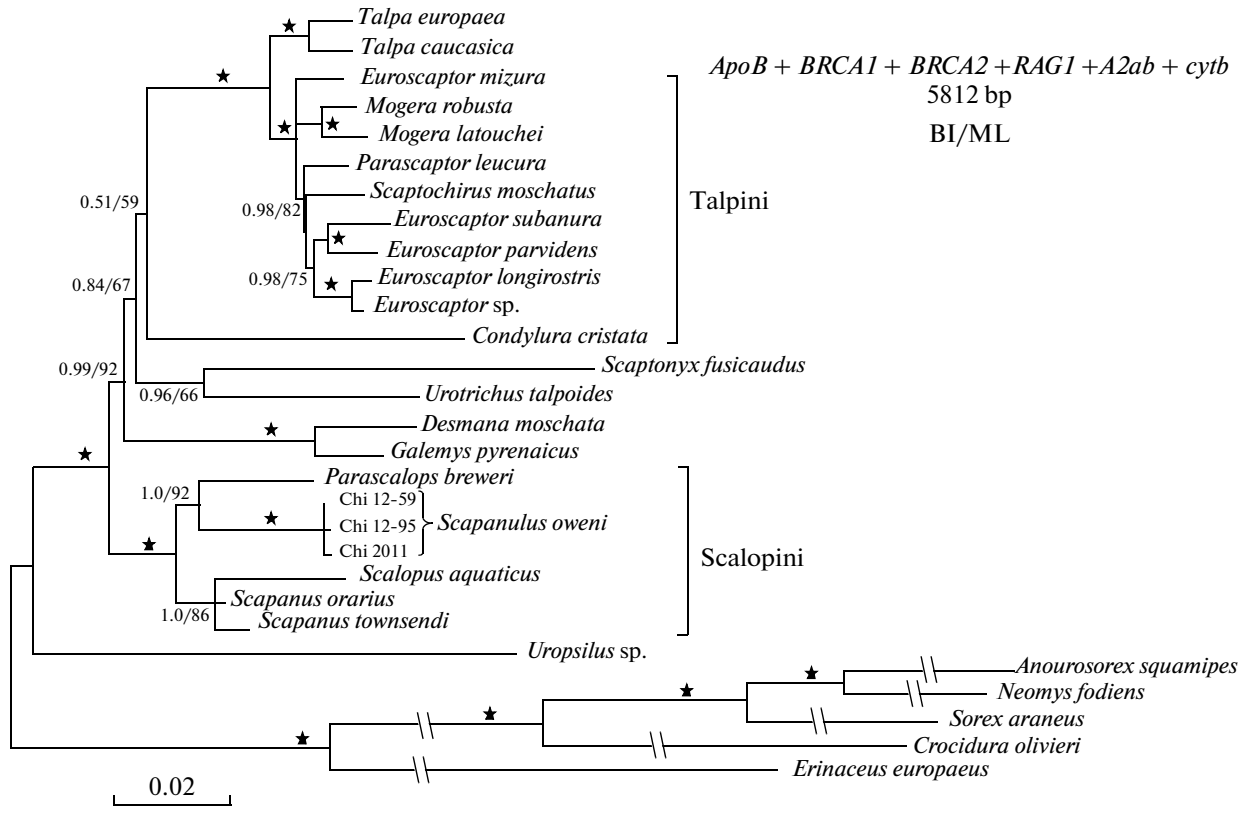

Fig. 2. The ML phylogeny of Talpidae as inferred from concatenated alignment of five nuclear genes (5052 bp) and conservative substitutions of *cytb* (760 bp: transversions at the 1st codon positions and all the substitutions at the 2nd codon positions). The designations as in Fig. 1.

pendent evolutionary models were used for each gene: for the 1st and 2nd positions combined and for the 3rd positions, respectively. The *cytb* data were analysed employing specific models for each of the codon positions.

At present, *cytb* sequences for all known genera of talpids are available from GenBank, however, the mitochondrial tree (Fig. 1) shows poor resolution and low support for most clades. The monophyly of Scalopini appears only as a tendency because of the lack of support for the association of the American representatives of the tribe with *S. oweni*.

The nuclear tree (data not shown) highlights two important results. First, the monophyly of Scalopini tribe including *Scapanulus*, is highly supported. Second, there is a lack of affinity between the two highly fossorial tribes, Talpini and Scalopini, which contradicts the morphological cladistic scheme [8]. The relationships within Scalopini could be resolved only by a combined analysis of mitochondrial and nuclear genes (Fig. 2). In this case the tribe is divided into two sister groups including *Scapanulus* + *Parascalops* and *Scalopus* + *Scapanus*, respectively, which fully corresponds to morphological data [7, 8]. In all other respects the topology of the combined nuclear-mitochondrial tree reproduced the one obtained with nuclear genes only.

All molecular studies [9, 10], including the present work as well as morphological data [7, 8, 10] consistently place the highly divergent genus of non-fossorial shrew moles *Uropsilus* as the basal lineage within the family. Short internal branches separating other tribes in the molecular tree (Fig. 2) may be suggestive of relatively rapid diversification among talpids, which makes it difficult to establish the precise order of branching events. As we move from the root down the phylogeny (Fig. 2) the tree shows successive separation of the specialized subterranean tribe Scalopini (with high support), semiaquatic Desmanini, semifossorial *Urotrichus* and *Scaptonyx*, and, finally, semifossorial-semiaquatic genus *Condylura* as the sister branch to the highly fossorial tribe Talpini. On the morphological scheme the tribe Urotrichini (*Urotrichus* + *Dymecodon*) branches off first after *Uropsilus*, being followed by Desmanini, *Neurotrichus*, and *Condylura*, which is placed as sister to the group comprising strictly fossorial Talpini and Scalopini and semi-fossorial *Scaptonyx fuscicaudus* [8]. Therefore, the main discrepancy between the morphological and molecular results stems from the fact that the latter reject close relationship between the two strictly fosso-

rial groups of moles—Talpini and Scalopini. The branching pattern of the molecular tree supports the hypothesis suggesting the convergent nature of morphological adaptations in Talpini and Scalopini, and their independent specialization to the subterranean life style [9, 12].

Divergence times in Scalopini were estimated based on the concatenation of nuclear and mitochondrial genes using the programme r8s version 1.8 [13]. Depending on the selected smoothing factor the radiation time of Scalopini varied from 26.81 to 20.25 Mya, whereas the divergence time of *Parascalops breweri* and *S. oweni* ranged from 21.85 to 16.78 Mya. When the smoothing factor selected by the cross-validation method was used, the results corresponded to more recent times (~20 Mya for Scalopini tribe and ~17 Mya for *S. oweni*).

The clarification of the phylogenetic position of the Gansu mole contributes to the understanding of the biogeographical history of the family. Based on fossil data the Talpidae is believed to have originated in Eurasia [14]. It is suggested, that Scalopini also has its origin in the Oligocene of Eurasia with independent radiations in both America and Asia [15]. The ancestors of North American genera are supposed to have migrated from Eurasia via a land bridge in the western Atlantic or the Bering Sea [6, 12], however, the number and geological time of these migrations remain unclear so far [6, 7, 12]. Earlier one migration into North America and two back migrations into Eurasia (including the ancestor of *S. oweni*) were hypothesized based on the parsimony analysis of morphological characters [8]. However, our molecular tree conforms to the scenario implying several independent colonizations of North America.

It is probable that morphological and molecular similarity of *S. oweni* and *P. breweri* reflect their genealogical proximity. Presumably, the ancestors of Scalopini have undergone extensive radiation in Eurasia. Afterwards both contemporary branches (*Parascalops* + *Scapanulus* and *Scalopus* + *Scapanus*) independently migrated into North America, whereas in Asia they were eventually replaced by other mole lineages (except of the Gansu mole). This hypothesis shows better correlation with palaeontology, because Scalopini appears relatively late in North American fossil record—only in the Middle Miocene [14]. Therefore, one may suggest that the Gansu mole is the last relic of Scalopini in Eurasia.

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