

# **The significance of acontia for the traditional classification of Actiniaria. Conflict of morphological systematics and modern opinions based on the study of molecular markers**

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**ABSTRACT:** In the order Actiniaria, one of the largest group of sea anemones Acontiarina was defined by Carlgren on the basis of the presence of special stinging organs — acontia. Inside it, the division into families was carried out according to a set of stinging capsules, which equip acontia. With the improvement of optical and electron microscopy, further study of stinging capsules led to a change in their formal classification, which violated the strictness of family diagnoses.

Nevertheless, this did not lead to an immediate restructure of the classification of acontiarian sea anemones, since other characters included by Carlgren in the diagnoses of the families were selected on the basis of a very thorough knowledge of the diversity of forms in this group and the deep intuition of this major specialist.

Published in recent years attempts to improve the system undertaken with the help of molecular-genetic methods, on the contrary, led to paradoxical results.

On the branches of phylogenetic trees, many closely related genera were isolated, and on schemes constructed using different markers (12S, 16S, 18S, 26S) they occupied very different positions. To clarify the reasons for the inconsistency of molecular data with traditional morphological classification, I conducted a pairwise comparison of the same sections of mtDNA. Pairwise comparison of the nucleotide sequences of mitochondrial genes showed the accumulation of multiple substitutions in some parts, indicative of a long independent evolution, and complete identity in other parts. These results, it seems to me, can be explained by the presence of recombination between the divergent regions of mtDNA and the unchanged regions, preserved in the nuclear genome of the cell in the form of *numts* — nuclear copies of mitochondrial DNA.

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**KEY WORDS:** acontiarian sea anemone classification, 12S, 16S, 18S, 26S, mtDNA recombination.

## **Значение аконтий для традиционной классификации Actiniaria. Конфликт морфологической систематики и современных взглядов, основанных на изучении молекулярных маркеров**

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**РЕЗЮМЕ:** В составе отряда Actiniaria одна из самых больших групп морских анемон Acontinaria была выделена Карлгреном на основании наличия специальных стрекательных органов — аконтий. Внутри нее деление на семейства было проведено по набору стрекательных капсул, вооружающих аконтии. По мере совершенствования оптической и электронной микроскопии дальнейшее изучение стрекательных капсул привело к изменению их формальной классификации, что нарушило строгость диагнозов семейств. Тем не менее это не привело к немедленной перестройке классификации аконтиарных морских анемон, поскольку другие признаки, включенные Карлгреном в диагнозы семейств были подобраны на основании весьма полного знания разнообразия форм и глубокой интуиции этого крупнейшего специалиста. Опубликованные в последние годы попытки усовершенствования системы, принятые с помощью молекулярно-генетических методов, напротив, привели к парадоксальным результатам. На ветвях филогенетических деревьев многие близкие роды актиний оказались разобщены, причем на схемах, построенных с использованием разных маркеров (12S, 16S, 18S, 26S) они заняли совершенно разное положение. Для выяснения причин несогласованности молекулярных данных и традиционных морфологических построений мной было проведено повторное, но на этот раз попарное сравнение тех же маркерных участков мтДНК. Попарное сравнение нуклеотидных последовательностей митохондриальных генов показало накопление множественных замен в одних их частях, свидетельствующее о длительной независимой эволюции, и полную идентичность в других частях. Эти результаты, как мне кажется, можно объяснить наличием рекомбинации между дивергировавшими за время независимой эволюции видов участками мтДНК и неизменными участками, сохранившимися в ядерном геноме клетки в виде *numts* — ядерных копий митохондриальной ДНК.

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**КЛЮЧЕВЫЕ СЛОВА:** классификация морских анемонов, 12S, 16S, 18S, 26S, mtDNA рекомбинация.

## Introduction

Up to date in the order Actiniaria there are a lot of closely related genera which without injury could be united, and a number of families which had been described mainly due to their geographical or bathymetric isolation. Formally, they have distinct morphological differences, nevertheless it is quite clear — where, to what genus and family, we could refer their species, being not disposed to recognise a new taxon. As an example for that genera *Bunodactis* and *Anthopleura*, *Actinauge* and *Phelliactis*, and also family Galatheanthemidae could be mentioned. The only genus of the family, *Galatheanthemum*, inhabits mainly deep-water trenches (in depths of 4000–10000 m and more). It could be surely put into family Halcampidae, if to neglect the unusually strong development of cuticle, which arises around the polyp's body a sort of abode, like the tube of sedentary polychaete worms. It should be pointed out that hypertrophy of cuticle occurs also in other groups of sea anemones — in *Stylobates* from family Actiniidae, in *Amphianthus dohrnii* from Hormathiidae, — but has not been considered there as a character of family level.

Except of those optional, “arbitrary” genera and families (which do not produce in fact any difficulties for taxonomists), and so called “good” families with original, sharply deviated characters, the system of Actiniaria includes some rich of species, slightly differentiated groups, which are difficult to divide into families and genera due to the great similarity of their members. Such groups could be interpreted as bushes of recent speciation or, to the contrary, it is possible to explain their morphological uniformity by the slower morphological divergency in old phylogenetic branches living in similar conditions.

In addition, considerable worry gives us a change in established views on the origin, resemblance, and homology of morphological structures, which were for a long time used as a basis of classification. Stubborn, conservatively disposed taxonomists can, after all, disregard the complicated speculations in the field of

comparative anatomy. They may believe that a time-tested, harmonious classification could itself serve as a good confirmation of the ideas laid down in its foundation. The worse situation comes if direct morphological study shows a deep, essential similarity of structures which earlier, under more superficial consideration, looked to be different. Quite the same situation took place in the study of acontia.

One of the largest groups of sea anemones, Acontiaria, was divided into families by Carlgren, the founder of the modern classification of Actiniaria, who divided this group into families, using differences in the set of nematocysts in acontia — specialised thread-like stinging organs located in the gastrovascular cavity. Not all sea anemones are supplied with acontia. Concerning to monophyletic origin of acontiate sea anemones in framework of order Actiniaria, the opinions of the authors vary. It could be rather said that it was Stephenson, who paid the most essential attention to the presence or absence of these organs. Primarily he believed that acontia were previously peculiar to all sea anemones with mesogloal sphincter (Mesomyaria), but later on they were lost in several branches (Stephenson, 1920: p. 558, 563). The closely related point of view holds Schmidt (1972).

In accordance to Carlgren's classification which was lately accepted by Stephenson, most of Actiniaria families supplied with acontia belong to sub-tribe Acontiaria (now renamed to superfamily Metridioidea) of infraorder Thenaria. Nevertheless, families Halcampactinidae (=Haliactiidae), Octineonidae and Andvakiidae were placed to infraorder Athenaria. Carlgren (1949) recognised that acontia were formed several times. Stephenson took more cautious but less clear position; he thought that all forms possessing of them were descended from a common ancestor (Stephenson, 1920: p. 444). Thus, the question of the independent origin of acontia in several groups of sea anemones was raised long ago and remained open for a long time. To solve similar, controversial questions of morphology and systematics, modern molecular-genetic methods have been increasingly attracted lately. Unfortunately, it should be noted that

in most cases “more modern” genetic characters (=markers) are now considered as more trustworthy than long-used morphological features.

In the manuals on molecular phylogenetics and in research articles, it was repeatedly emphasized that the proposed trees reflect primarily the similarity of genes (compared nucleotide and amino acid sequences), on the basis of which they were constructed, but not the relationships of the studied organisms. Description of the morphology of animals also reveals only a similarity, which does not always indicate a relationship. It may be associated with similar environment or simply with common patterns of morphological evolution. Similarity in molecular markers, it would seem, should yield more reliable results, since genetic “texts” are not subjected to direct control of the environment and selection. They only accumulate with time the random (non-adaptive) differences that we find in the divergent phyletic lines.

## Results and discussion

The attempt to distinguish between the actinians, that acquired acontia as a result of independent evolution, from the really closest and related families, led to unexpected results.

A number of appreciated specialists who have done a lot in their earlier works to improve the morphological systematics, have undertaken a comparison of many acontiate sea anemones with methods of molecular genetics (Daly et al., 2010; Rodriguez, Daly, 2010; Rodriguez et al., 2014). They used mitochondrial (12S, 16S, cytochrome oxidase subunit III) and nuclear ribosomal RNA genes (18S and 26S) as markers. Representatives of the diverse, including distant branches of the genealogical “tree” or, more correctly, the “bush” of order Actiniaria were investigated. In the trees constructed using four ribosomal RNA genes, the acontiate genera *Sagartia*, *Metridium*, *Hormathia*, *Calliactis* and others occupied a very different position (see Daly et al., 2010: figs. 4a, 4b, 4c, and 4d). It must be acknowledged that similar problems were encountered by specialists who carried out revision of many other groups, for example, the

genus *Acropora* (Scleractinia, Cnidaria) (Open et al., 2001).

Let’s try to see — what, in fact, is the difference between the sequences belonging to the ribosomal genes that lie in the mitochondria of both related and not closely related genera. For our comparison, the same sequences of genes 12S and 16S were used that were analysed by our predecessors<sup>1</sup>. Pairwise alignments of every couple of genes were performed with the programs “Blast” and “MEGA-7”. They contain many nucleotide substitutions (mostly SNPs, single nucleotide polymorphisms) that should have accumulated from the moment of divergence and the independent existence of genera. These replacements are nevertheless distributed in the body of the ribosomal genes unevenly. Almost along the entire length, there are from 2 to 10 substitutions for a hundred nucleotides. But there are long sections of some hundreds nucleotides, which in the compared anemones show a complete identity. In this case, it can not be said that a pairwise comparison made it possible to find mutable or, on the contrary, conservative sites. In *Metridium* and *Nematostella* the perfect similarity is shown in other parts than in *Nematostella* and *Sagartia*.

As we see, the nucleotide sequences of ribosomal genes consist of unlike sections (hence, long separated, altered by time), and sections that are completely unchanged, as if belonging to the same species. The same picture is shown also for complete mtDNA, read through so far only for a few species of sea anemones. It seems to me that this phenomenon can only be explained by combining unlike and unchanged pieces of mtDNA, derived from different sources.

The exchange of large sections of DNA, as we used to think, in all living beings, from bacteria to humans, is carried out during the sexual process, which ensures the exchange of homologous sequences. However, the cross-breeding that occurs during sexual reproduction is possible only between closely related animals, but not between the sea anemones of different families.

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<sup>1</sup> Accession numbers see — <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0010958>

Another form of recombination of genetic material — the so-called horizontal gene transfer — unlike sexual reproduction does not require close affinity and plays a massive role in the evolution of prokaryotic organisms (Shestakov, 2009; Ravin, Shestakov, 2013). Horizontal gene transfer is also common among microbial eukaryotes (Andersson, 2005), but in multicellular organisms this phenomenon is rare, exotic. Nevertheless, to date, it has been proven that horizontal gene transfer is possible between the non-close groups of eukaryotic multicellular animals. Apparently, it is responsible for the “gene recruitment” of transfer RNA in the mitochondrial genome of Demospongia and even in monkeys (Wang, Lavrov, 2011). In addition, horizontal gene transfer is observed in populations of bdelloid rotifers that maintain a high level of genetic polymorphism in their populations by borrowing foreign genes that appear to have originated in bacteria, fungi, and plants (Gladyshev et al., 2008).

Finally, another one source of DNA, highly homologous to mitochondrial genes, is located in the nucleus of cell. *Numts* — nuclear sequence of mitochondrial origin — were originally found in the migratory locust (Gellissen et al., 1983). To date, they have been searched out in the nuclear genome of felines, apes, humans and very many other organisms. According to later works (Kim et al., 2006; Hazkani-Covo et al., 2010), DNA copies stored in the nucleus are able to change many times slower than DNA working in mitochondria. In this connection, they are reasonably considered as “molecular fossils”, which can be used for genealogical reconstructions. That is why it seems to me most sensible to explain the presence of identical nucleotide sequences in the mitochondrial genome of remote species of sea anemones not by horizontal gene transfer but by the exchange between working mtDNA and copies of the mtDNA of their old ancestor buried in the nuclear genome in the form of *numts*. The main objection to this interpretation remains the concept of the clonal inheritance of mitochondrial DNA, which was acquired many years ago.

Recombination of mtDNA has for many years been the subject of debate between researchers. On the one hand, the mathematical analysis of a rich array of sequences, performed by authoritative specialists, led to the conclusion about the existence of recombination (Eyre-Walker et al., 1999; Eyre-Walker, 2000; Bromham et al., 2003; Tsaousis et al., 2005). On the other hand, our complete misunderstanding of the mysterious mechanism capable of replacing one mtDNA haplotype with another in all cells and in all mitochondria of the multicellular organism does not allow us to abandon the habitual delusion. Until now, the notion of strict matrilineal transmission of mitochondrial genetic markers has been accepted by most specialists involved in molecular phylogenetics. However, the inconsistency of the results obtained with the use of mitochondrial and nuclear ribosomal genes, as we saw in sea anemones, makes us doubt the correctness of these deep-rooted views.

In any case, the independent evolution of 12S and 16S genes, which is visible in the dissimilarity of the phylogenetic trees constructed on their basis, can apparently be considered an argument against their coherent inheritance and an important evidence in favor of mtDNA recombination.

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