ISSN 2079-0864, Biology Bulletin Reviews, 2019, Vol. 9, No. 4, pp. 275–294. © Pleiades Publishing, Ltd., 2019.
Russian Text © The Author(s), 2019, published in Uspekhi Sovremennoi Biologii, 2019, Vol. 139, No. 1, pp. 3–24.

Estimates of Genetic Introgression, Gene Tree Reticulation, Taxon Divergence, and Sustainability of DNA Barcoding Based on Genetic Molecular Markers

Yu. Ph. Kartavtsev[a, b, *] and A. D. Redin[a, b]

[a] *National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, 690041 Russia*

[b] *Far Eastern Federal University, Vladivostok, 690091 Russia*

**e-mail: yuri.kartavtsev48@hotmail.com*

Received July 5, 2018; revised July 25, 2018; accepted July 25, 2018

Abstract—The evidence for the possible impact of gene introgression on species evolution, the evolutionary fate of taxa, including reticulations in phylogenetic trees, and the consistency of the latest molecular genetic data with the main modern paradigm, Neo-Darwinism, have been considered in many studies. This study includes a comparative analysis and assessments of validity of the use of molecular markers for species identification, including an approach proposed by one of the authors for the description of biological diversity in the framework of the global DNA barcoding program. The identification of hybrids and the prevalence of genetic introgression are discussed. There are four main issues in the overview presented. (1) A combination of nDNA and mtDNA markers best suits for the hybrid identification and estimates of genetic introgression or gene flow. (2) The available facts for both nDNA and mtDNA diversity make introgression among many animal and plant taxa obvious, although, even for the wide hybrid zones of *Mytilus* ex. group *edulis*, for example, introgression may be quite restricted or asymmetric for a significant part of the area, thus holding at least the "source" taxon (taxa) intact. (3) If we accept that sexually reproducing species in marine and terrestrial areas are introgressed, as is evident for many cases, then we should recognize that the orthodox BSC, which postulates a complete lack of gene flow among species, is inadequate due to the fact that many zoological or taxonomic species have currently not yet reached the stage of biological species. However, they will eventually become definitive biological species in future. This conclusion is supported by the genetic distance, which increases with taxa rank, and by the lowest diversity at the intraspecies level as for single mtDNA genes, for complete mitogenomes, and for nDNA genes. (4) A recent study of fish-taxon divergence by means of the vast BOLD (www.boldsystem.org) database showed that the gene trees for taxa up to the family level are basically monophyletic, and interspecies reticulations are rare for most gene trees. The available data allow us to conclude that molecular evolution and, in particular, genetic divergence in the taxon hierarchy are generally in good agreement with BSC and Neo-Darwinism, forming the theoretical basis for the success of DNA barcoding in the animal world, as well as its applicability for other organisms.

Keywords: DNA barcoding, molecular evolution, genetic distances, nucleotide diversity, genetics of speciation, *Co-1*, *Cyt-b*, nDNA, mtDNA

DOI: 10.1134/S2079086419040042

INTRODUCTION

DNA barcoding has been widely used in biology since 2003 (Hebert et al., 2003a, 2003b). However, the origins of the application of the variability of biological macromolecules, for example, to systematics and evolutionary biology have a long history (Antonov and Belozersky, 1961; Antonov et al., 1971; Zuckerkandl and Pauling, 1965; Hubby and Throckmorton, 1965; Nanney, 1982). In general, biological molecular markers have found numerous applications depending on the needs of modern society. A biological molecular marker (MM) is any macromolecule of a living organism that can be an identifier for a particular function, the results of a biochemical, population, or evolutionary process. The study and use of MMs have already become a new branch of biomedical science, as evidenced by the presence of special journals (*Biomarkers*, *Journal of Current Biomarker Findings*, *Biomarker Insights*, *DNA Barcodes*, etc.). MMs are used in biology and medicine in many areas. There are three most important areas.

(1) DNA barcoding. MMs have found application in the global program for the redescription of biological diversity on a modern molecular and bioinformational basis (iBOL, international barcode of life project; www.ibol.org). For most invertebrates and verte-

brates, a nucleotide sequence (hereafter, the sequence) of the *Co-1* gene, which encodes cytochrome *c* oxidase subunit 1 of mitochondrial DNA (mtDNA), is used as a standard marker or DNA barcode. For ease of operation, the first half of a gene with a length of about 650 base pairs (bp) is used as a barcode. Other MMs or barcodes are more suitable for plants (Schneer and Rodionov, 2018; Zhokhova et al., 2019). The basis for the successful identification of studied eukaryotic species is low intraspecific variability (weak sequence differences between specimens of the same species) but an intraspecies divergence of samples that is an order of magnitude higher (between specimens of different species). The average interspecific divergence is about 0.5–1.0 while interspecies it is 10%, according to data on animals (Kartavtsev and Lee, 2006; Kartavtsev, 2009a, 2011a, 2011b, 2013a, 2013b, 2013c; Stoeckle and Thaler, 2018, Fig. 2).

(2) MMs for the identification of stocks, lines, and breeds of animals. For this level, *Co-1* and other mtDNA MMs are not quite suitable because they have relatively low variation within the species (although there are exceptions); MMs of nuclear DNA (nDNA) usually are more conservative in animals and even less applicable at this level. The highest effectiveness for the identification of differences between animal populations, breeds, and lines and to authenticate single individuals in higher organisms is manifested by microsatellite DNA loci and single nucleotide substitutions.

One MM application is the identification of hybrids and invasive species. Due to globalization and the intensification of international trade in food products, the identification of specimens of export–import operations is of great importance. Falsified trademarks, such as fish fillets, caviar, and other products, can be accurately determined with MMs, which helps public and private enterprises avoid significant economical and reputational losses (Nedunoori et al., 2017; etc.).

(3) The greatest importance of MM lies in disease diagnostics in medicine (in particular, breast cancer, prostate cancer, colon cancer, etc.) and the exclusion of certain specimens from suspects in criminalistics (forensic medicine). The scope of MM includes the monitoring of genetic safety to assess the risks of the use of recombinant DNA and genetically modified products/objects in the food and medical industries (Zhokhova et al., 2019).

The areas of study described above, besides the obvious ones—medicine and biodiversity—are particularly important for the paradigms of general biology, evolutionary genetics, and the scientific component of the iBOL program. On June 21, 2017, the iBOL database contained the results of studies of 8452436 specimens of living organisms. The number of specimens with barcodes is 6108027, and the number of species identified by barcodes is 279679 (http://v4.boldsystems.org/in-dex.php/TaxBrowser_Home). All of these data are accompanied by unified documentation that follows iBOL standards, and they are available to any user via the Internet. The contribution of the Russian Federation and RUS-BOL (http://www.imb.dvo.ru/misc/barcoding/index.htm) to DNA barcoding research is reflected in the centralized database BOLD (barcode of life database) (www.boldsystems.org) and includes 32177 records published in the database, forming 7097 barcode clusters represented by 241 organizations (laboratories). The records made in BOLD refer to 16764 species names, representing 4438 species. According to the activity, the Russian Federation is located in the middle of the list of program participants, at the same level as Brazil and France.

Species identification based on DNA barcodes has been successful for the vast majority of taxa. It now forms an extensive natural science basis and requires explanation and theoretical justification (Kartavtsev, 2013a, 2018; Stoeckle and Thaler, 2018). In one approach to the representation of the biological basis of this phenomenon, it was proposed to focus primarily on the "pairwise distance" metric, which is equivalent to the *p* distance or fraction of different nucleotides in a pair of randomly selected sequences (Nei and Kumar, 2000; Kartavtsev, 2011a, 2013a), and to evaluate the molecular features of *Co-1* and mtDNA as a whole (Stoeckle and Thaler, 2018). In another approach, which is implemented in this study, it was assumed that this feature exists mainly due to the prevalence of the geographical mode of speciation in nature, which allows organisms to accumulate stochastic mutations and unique nucleotide substitutions in DNA chains and other macromolecules during the formation of daughter populations (taxa) under isolation conditions. With the implementation of this model, specimens of different species are experimentally identifiable by DNA barcodes, and a correlation of *p* distances and taxon ranks can be detected by the appropriate analysis (Kartavtsev et al., 2011a, 2011b; Kartavtsev, 2013a, 2013c).

In order to understand these questions, special consideration of empirical data, with diversified analysis and conjugation with genetic bases of speciation, as well as with the relevant provisions of the Biological Species Concept (BSC) and, more generally, with Neo-Darwinism, or Synthetic Theory of Evolution (STE), are required. The relevance of this study is also determined by the need to consider criticism of the BSC/STE paradigms based on the concepts of extensive introgression (Arnold, 1997, 2008; Arnold and Emms, 1998; Arnold and Fogarty, 2009) and reticulative evolution (Arnold and Fogarty, 2009; Borkin and Litvinchuk, 2013). These questions have already been partially considered (Kartavtsev, 2005, 2009a, 2011a, 2011b, 2013a, 2013b, 2013c).

This study presents a joint review of introgression and reticulation, as well as an analysis of genetic distances for MMs of *16S* rRNA and for complete mitochondrial genomes (mitogenomes) for sequences from the GenBank (www.ncbi.gov). These markers have not been used previously in a comparative-and-evolutionary aspect. The variability of genetic distances in the taxon hierarchy for the *16S* rRNA gene is not presented in the literature, and it was previously considered only for three specific taxa for mitogenomes (Kartavtsev et al., 2016; Turanov et al., 2016; Redin and Kartavtsev, 2017). One key objective of this brief review is to answer the question of whether the available molecular genetic data allow generalizations about the wide presence of genetic introgression and reticulation in the studied gene trees, or, conversely, whether they are consistent with BSC/STE. STE itself is certainly not a dogma and requires further development. In fact, in biology, STE is a general evolutionary concept and therefore may be referred to as a theory. However, based on the formal scientific position, it is unlikely to meet the criteria of a theory. A theory should contain an analytical description in mathematical terms and/or should represent a rigorous model, e.g., a computer model, and have predictive components. An understanding of this deficiency is available in the relevant literature, and fragments of theoretical studies with different level of details appear periodically (Bush, 1975; Nei, 1987; Templeton, 1981, 1998; Kondrashov, 1998; Zhuravlev and Avetisov, 2006; Kartavtsev, 2009a, 2009b, 2011a, 2011b, 2013a, 2015).

Data on the possible influence of gene introgression on species evolution, the evolutionary fate of taxa, including reticulations of phylogenetic trees, and the consistency of modern molecular genetic data in general with the main modern evolutionary paradigm, neo-Darwinism, have been presented in many publications (Barton and Hewitt, 1985; Campton, 1987; Arnold, 1997, 2008; Avise, 2000; Gerber et al., 2001; Arnold and Fogarty, 2009; Kartavtsev, 2013b; Stoeckle and Thaler, 2018). Considering the problems of the review, it is important to clarify some phylogenetic terminology at the very beginning of the article. The term "gene tree" was introduced a long time ago (Tateno et al., 1982; Nei, 1987; Kartavtsev, 2009a). A gene tree is a phylogenetic tree constructed from data for a single gene. This term is the opposite of the concept of a species tree (Nei, 1987; Kartavtsev, 2009a, p. 189), which includes a phylogenetic signal for several genes and may incorporate other traits. A phylogenetic tree, including a gene tree, may have a different topology, including common roots for branches/nodes/clusters (monophyly) or other branches (para or polyphyly). There are various controversial issues regarding the BSC/STE. This review focuses mainly on four questions: (1) What detection methods for hybrids and genetic introgression, or gene flow, are the most appropriate? (2) What do the facts obtained based on markers of mtDNA and nDNA indicate for BSC/STE? (3) Is there evidence in the literature on the consistency of molecular variability in phyletic lines or taxa with BSC/STE? (4) How often do reticulations and polytomies of gene trees occur, and what is the main information signal revealed by their topology? The article primarily analyzes data for animal taxa, but many ideas may apply also to other groups of organisms.

CONCEPT, TERMS, AND METHODS FOR ADEQUATE EVALUATION OF GENETIC INTROGRESSION

Hybrids and Hybridization: Clarification of This and Related Terminology

Two concepts are important for an understanding of the essence of genetic introgression: hybrid and hybridization. A hybrid is a genetic mixture or offspring from a crossing between genetically different organisms. A specimen with a mixed pedigree, a mestizo, can also be considered a hybrid. The limited case of a heterozygote for one or more loci is a hybrid. Thus, crossing P_1: $A_1A_1\ B_2B_2 \times A_2A_2\ B_3B_3$ provides the hybrid $F_1 = F_H$: $A_1A_2\ B_2B_3$. Hybridization is the process by which hybrids appear. Meanwhile, the difference between simple intrapopulation crossing and the crossing of specimens of different lines, populations, and species should be recognized. In the usual sense, hybrids are consider as descendants of more distant crosses. Moreover, the crossing distance is a conditional concept that depends on the species of organisms and the normal system of breedings that have developed for this organism. In addition to F_1, other types of hybrids can occur: $F_1 \times F_1 = F_2$, $F_1 \times P_1 = F_b$, etc.

Hybridization can be artificial or natural. This article focuses primarily on natural hybridization. As noted above, it is important to understand hybridization and hybrids as genetic entities that are defined in terms of genotypes and crosses in the natural environment, normally between cross-breeding organisms. Agamic and clonal forms were not considered in this study.

Hybrids do not necessarily have to be intermediate by phenotype between the parental forms, but, depending on the complexity of the crossing, they may be closer to one of the parents and in nature are usually less adapted than the parents. Accordingly, the hybrid index, e.g., I_H (Campton, 1987), may be far from 0.5 (Kartavtsev, 2009a, 2015, ch. 10). During artificial reproduction and linear selection, even the opposite effect is possible with the emergence of heterosis in hybrids of distant crosses between inbred lines or certain types of breeds and varieties. However, in natural populations, excess variability often does not have a positive effect, providing an additional genetic load (Kartavtsev, 2009a).

It is necessary to clarify a few more terms. First, the gene flow is a process marked by selectively neutral (or

nearly neutral) alleles. Reproductive isolation between biological species indicates the absence of any type of gene flow. When there are no hybrids or when F_1 and especially F_2 or F_b are not fertile or not viable (low fertile, poorly viable), then there is no gene flow or it is negligible. On the contrary, the presence of a significant proportion of F_2 or F_b, and not F_1, should be considered as an indicator of gene flow. This formulation fits with the strict or orthodox BSC (Dobzhansky, 1955; Timofeev-Resovky et al., 1977; Mayr, 1982). Second, let us assume that the hybrid zone is a geographical space where hybrids of natural origin occur between the supposed parental forms. A cline often occurs in such zone. Third, we assume that a cline is a gradual (gradient) or abrupt change in the frequencies of alleles in the forward or reverse direction, type 1 (population 1) $\rightarrow$ hybrids $\rightarrow$ type 2 (population 2), supported by the balance between the distribution and selection against hybrids (modified from Barton and Hewitt, 1985).

From the definitions presented above, it follows that the exact determination of the presence of hybrids is possible with the use of nuclear gene markers, which make it possible to identify the alleles of both parents in the genotype. Markers of mtDNA, which are normally inherited in most animals as components of the oocyte cell plasma, e.g. maternally, can be used, but only under certain conditions. Thus, the presence of a fragment of mtDNA of type X or a complete mitogenome in the study of type Y in samples can be explained by a hybridization event that occurred in the past. However, it is necessary to exclude such events as horizontal transfer, recombination, etc. Evidence of the presence of hybrids obtained only with mtDNA markers should be considered as preliminary, since, as defined above, the hybrid genotype can be accurately identified only by the nuclear genome. Such evidence may sometimes reflect the recombination of a region of the mtDNA gene with nDNA. For example, such events were observed in carp fish of *Gila robusta* complex (Gerber et al., 2001). The transfer of mtDNA from *Brachymystax lenok* in *Hucho taimen* were also described (Balakirev et al., 2013). Both of these examples are only preliminary indices of possible hybridization, since they do not contain data for nDNA markers.

Descriptions showing that taxonomically different fish can interbreed and produce fertile offspring are well documented (Hubbs, 1955). Researchers (Schwartz, 1972, 1981; Barley et al., 2001; Altukhov et al., 1997) summarized data from more than 4000 studies with examples of artificial and natural fish hybridization, but genotypic documentation was not done in most cases. Some data on this topic were also presented in the study (Kartavtsev, 2013b).

It is believed that natural hybridization is more common in fish than in other vertebrates. A similar conclusion may be applied to marine invertebrates. The chromosome sex determination system, which is not as well developed as, for example, in mammals, should be the most common of the reasons for more frequent hybridization in these groups. In a majority of vertebrates, the sex is determined by a determinative gene (Devlin and Nagahama, 2002) located on the Y chromosome. In fish, sex is determined by several causes and only occasionally by sex chromosomes, which may be completely absent (Kirpichnikov, 1979). Recent molecular studies revealed a weak correlation between the phenotype of males and females with sex-specific genetic markers in salmon (Podlesnykh et al., 2017). The increased hybridization level in these taxa can be based on several features of the biology of fish and invertebrates: external fertilization, weak behavioral isolating mechanisms, unequal numbers of two potential parental species, competition for limited spawning biotopes, and, lastly, susceptibility to secondary contact of recently diverged forms (Avise and Sanders, 1984; Campton, 1987; Avise, 2001). These features can vary significantly depending on local conditions. Natural and human-induced changes in the external environment are often cited as factors that promote fish hybridization (Altukhov et al., 1997). For example, hybridization is relatively common in freshwater fish from temperate latitudes, where geological and climatic conditions have changed dramatically since the Pleistocene, transforming the freshwater environment while the marine environment remained relatively stable (Campton, 1987). Civilization-induced changes in ecosystems in North America also correlated with increased hybridization between the initially allopatric and naturally sympatric pairs of species (Hubbs et al., 1953; Nelson, 1966, 1973; Stevenson and Buchanon, 1973). For salmonid fishes, such examples were summed up separately (Simon and Nobble, 1968; Altukhov and Salmenkova, 1991; Altukhov et al., 1997). Available estimates indicate that hybridization affects the fate of about 25% of plant species and up to 10% of animal species (Arnold, 1997). It is assumed that cases of hybridization and subsequent genetic introgression usually occur among young, recently diverged species. As noted earlier, the focus will be on species that presumably better satisfy BSC provisions in the original understanding of the species (Mayr, 1982) or its versions (Timofeev-Resovsky et al., 1977; Kartavtsev, 2009a, p. 86; 2015, p. 95). Related publications considered environmental aspects (Genovart, 2008), recent historical changes (Seehausen, 2004), and the frequency of occurrence of natural hybrids in various taxa: for example, in birds (Grant, P.R. and Grant, R.B., 1992) as compared with other vertebrates (Prager and Wilson, 1975; Fitzpatrick, 2004).

Methods Used for Hybrid Identification

There are at least four methods for the identification of hybrids: (1) morphological, (2) karyological, (3) biochemical-genetic, (4) molecular-genetic

(Campton, 1987; Kartavtsev, 2009a, 2015). In principle, methods 3 and 4 can be combined into one method, since both relate to MMs. The first two methods were described earlier (Kartavtsev, 2009a, 2015) and will be omitted in the review for brevity, although both can be informative in terms of the possible presence of hybrids in populations. However, the first can only be considered a method that provides indirect evidence of the presence of hybridization, and the second is difficult to use due to the small chromosome size in many groups of organisms and the complexity of the techniques. However, chromosomal methods have been successfully used repeatedly in environmental protection studies (Setzer, 1970; Greenfield, D.W. and Greenfield, T., 1972; Greenfield et al., 1973; Busack et al., 1980; Phillips and Zajicek, 1982; Delaney and Bloom, 1984; Phillips et al., 1985; Vasiliev, 1985; Frolov, 2000).

MMs most fully meet the requirements of hybrid identification and are used to answer the question of the existence of genetic introgression. The determination of the facts of hybridization and introgression according to such MMs as allozymes and nDNA is relatively accurate if the two parent ancestors had different monomorphic (fixed) alleles for one or more loci. For example, for two X and Y loci with two alleles in each, X_1 and X_2 plus Y_2 and Y_3, the hybrid specimen F_H: X_1X_2 Y_2Y_3 is uniquely distinct from parental forms, since both parental forms have different homozygous genotypes, and the hybrid is represented by a heterozygous genotype for all diagnostic loci. However, if the hybridization continues beyond the F_1 generation, the progeny will have a wide range of recombinant genotypes, including genotypes identical to both parental forms. Accordingly, a specimen with a composite genotype that is identical by genotype to one of the parental forms can be F_1 hybrid obtained by self-crossing or by back-crossing with one of the parents.

In other words, the presence of recombinant genotypes can be recognized as evidence of the reproduction capability of hybrids and the presence of second-generation hybrids (F_2 or F_b). In such situations, it becomes absolutely necessary to ascertain the characteristics of genotypic variability in population-genetic terms, with an assessment of the parameters of migration, N_m and m, differentiation, F_{st}, etc. There are few studies in the existing literature that use such accurate population-genetic approaches, but the reviews reflect a wide range of other examples are available (Arnold and Fogarty, 2009; Borkin and Litvinchuk, 2013). A brief review of some of the conclusions of these studies will be provided below.

Empirical data confirm that allozymes and nDNA markers are most effective when the parents have different fixed alleles (Campton, 1987; Kartavtsev, 2009a, 2015; Kartavtsev et al., 2018b) and/or when multigenomic data (Fong and Chen, 2010; Nevado et al., 2011) with estimates of parameters such as N_m, m, F_{st} were analyzed. Combined approaches, e.g., the use of mtDNA and nDNA, can be even more successful, because they reveal, for example, the direction of parent's sex in mating due to the prevalence of maternal mtDNA inheritance in animals (Avise, 2001). An integrated approach using MMs and morphometry makes it possible to assess the presence of genotypic effects, in particular heterozygosity, on the phenotype (Kartavtsev et al., 2005, 2018b), which can be useful for mariculture. Other examples of the successful use of MM combination were presented for turtles of the genus *Mauremys* (Fong and Chen, 2010), for cichlids of the genus *Ophthalmotilapia* from the Lake Tanganyika (Nevado et al., 2011), for mussels of the *Mytilus* ex group *edulis* complex with *GLU-5* and other MMs (Heath et al., 1995; Inoue et al., 1995; Skurikhina et al., 2001; Kartavtsev, 2009a, 2015; Kartavtsev et al., 2014, 2018b), and also for some other taxa (Avise, 2001).

To summarize the data above, the following should be noted.

(1) Hybrid identification and the detection of the presence of genetic introgression are difficult tasks. First, these tasks require an accurate genetic analysis with hybrid identification based on many loci and a comparison of descendants of various types (F_1, F_2, F_b, etc.). Subsequently, estimates of the frequencies of alleles, the gene flow, and a generalization of all of the isolated components should be obtained.

(2) In this context, BSC is the basic concept for the selective testing of groups of organisms in genetic terms; inbred lines and agamic species (lines of organisms) cannot be considered representative for an understanding of the essence of these processes.

(3) The experimental tools available in genetics for the analysis are direct and sufficient for hybrid identification and assessment of the introgression level.

Implementation of Genetic Introgression via Species Barriers

It is often difficult or even impossible to detect hybrids if the hybridization is successful through generations and the progeny of the hybridization contains F_1, F_2, F_b, etc. In a mixed population, if descendants of reciprocal crosses or hybrids subsequent to F_1 generations are common, the presence of recombinant genotypes with a sufficiently high frequency is possible, and it becomes difficult to distinguish F_1 hybrids from rare parental heterozygotes, even with the use of several diagnostic nuclear markers (Campton, 1987). There are specialized software, such as Structure (http://pritch.bsd.uchicago.edu/structure.html), etc., which help to solve some problems in hybrid identification for a high amount of data. However, it should be noted that the subject of the study and numerical simulation are so complex that it is difficult to find an unambiguous, population-genetic solution for mass

hybridization. Especially difficult is the assessment of the genetic introgression level and its consequences over time. One difficulty is the lack of knowledge as to whether the analyzed hybridization forms have reached species status. This concerns the situation in which hybridization is detected, but the researcher does not have convincing data on the taxonomic rank of the objects, which in this case may have different species names. In this regard, the precise delimitation of species is a very important challenge facing modern evolutionary genetics and evolutionary biology in general (Brower, 1999; Sites and Marshall, 2004; Kartavtsev, 2009a, 2009b, 2011a, 2011b, 2015). To investigate this very complex subject, we implemented an approach that makes it possible to test the congruence of two information groups of data in BOLD. The first group contains materials from zoological collections (with samples assigned to certain taxa: species, genera and families), and the second group consists of specimens identified with MMs, more precisely, based on DNA barcodes (Ratnasingham and Hebert, 2007), with the assignment of a unique identifier (cipher) or a special index, called BIN (barcode index number) to each specimen.

One of the first studies of the frequency of occurrence of hybrids in natural populations was performed on aquatic biota: fish and invertebrates. In the 1980s mtDNA in combination with allozyme loci were used to study the frequency of hybridization between nine species of sunfish (genus *Lepomis*) inhabiting two geographic locality in the southeastern of the United States (Avise and Saunders, 1984). The researchers came to the following conclusions. (1) Hybridization occurs at a relatively low frequency, but five of the nine studied species were involved. (2) Neither mtDNA nor allozyme evidence was found for the existence of gene introgression between species of the genus *Lepomis*; all hybrids were exclusively F_1 offspring. (3) Each detected hybrid was a cross between the most common and the rarest species. (4) In six of seven possible hybrid finds, the parent was a representative of a rare species as determined by the mtDNA genotype. This feature was explained by intense competition among males for mating partners and the general promiscuity of females. Since then, the number of studies in which cases of interspecific hybridization in nature were studied increased significantly. Most of them demonstrated the presence of only F_1 hybrids in natural populations. The hybrids of several subsequent generations and the descendants of backcrosses, through which introgression of alien genes into the genomes of parental species occurs, were less common (Avise, 2001).

Examples of mtDNA analysis can be easily expanded. Some will be discussed below. In particular, sea mussels (Mollusca, Mytilidae) were often used for the study of hybrid zones. We will consider some of our own data for the Sea of Japan related to the Pacific mussel *Mytilus trossulus* and to the invasive Atlantic species *M. galloprovincialis*. In an earlier study, a combined allozyme-and-morphometric approach was used; it revealed approximately 5% of hybrids in the waters of Russia, South Korea, and Japan, with their share varying from year to year from $1.6 \pm 0.9\%$ to $8.9 \pm 1.7\%$ (Kartavtsev et al., 2005). Based on data on the prevalence of gene flow in the *M. trossulus* → *M. edulis* → *M. galloprovincialis* direction, the authors suggested that the species rank of *M. trossulus* in the *Mytilus* ex. group *edulis* complex was achieved. At the same time, *M. edulis* and *M. galloprovincialis* taxa should be considered in the rank of subspecies/semispecies if we follow the definition of the orthodox BSC (Kartavtsev et al., 2005). This conclusion is in good agreement with paleontological dating, according to which *M. trossulus* is the most ancient element of the *Mytilus* ex. group *edulis* complex (Kafanov, 1987; Kartavtsev et al., 2005), and with molecular phylogenetic estimates of divergence in the family Mytilidae (Kartavtsev et al., 2018a). Recent studies have again presented a comprehensive population-genetic and morphometric study of mussels of this group from the northwestern part of the Sea of Japan (Kartavtsev et al., 2014, 2018b). Specimens were genotyped with eight polymorphic enzyme loci and two nDNA markers (*GLU-5* and *ITS-1,2*). Enzyme and nuclear markers detected consistent genetic variation in the frequencies of the parent species and their hybrids. The genotypes of the local species *M. trossulus* predominate in the studied samples, and hybrids were found with a relatively low frequency (Fig. 1). The total frequency of invasive *M. galloprovincialis* species was relatively low, but it reached a value of $42 \pm 2\%$ in one of the samples in Posyet Bay, near the village of Zarubino, which is also a port that receives ships of the international passenger line Russia–Korea. The largest number of hybrids was also found in this region. A joint analysis of genetic and morphological variability revealed a generally low differentiation among mussel populations. The exchange between populations in the surveyed region expressed as the flow of migrants per generation (N_m) was $N_m = 5$ (Kartavtsev et al., 2018b). The proportion of migrants, assuming that the progeny of F_2, F_3 and F_b, rather than F_1, that mainly provide the interspecific gene flow, was estimated as $F_b + F_2$ and was equal $0.9 \pm 0.7\%$. The gene flow for a wider region of comparison in the Sea of Japan was previously estimated as $N_m = 0.5–2.0$ (Kartavtsev et al., 2005). Thus, the obtained data proved the persistence of *M. galloprovincialis* invasion in the northwestern part of the Sea of Japan. Based on the occurrence of hybrids of all types, it can be assumed that the level of genetic introgression between two taxa in the region was low, varying over a 14-year observation period in Vostok Bay (Peter the Great Bay, Sea of Japan). The variations had the following range: 0% in 2012 and 2013 (Kartavtsev et al., 2018b) and $8.95 \pm 1.68\%$ in 1999 (Skurikhina et al., 2001). The data sup-

ports the concept of a bimodal hybrid zone with a limited hybridization level between the two nominal species in the contact zone. In other areas of the range, the situation may differ. However, the recombination level, for example, which is based on the *MPI** allozyme locus, was higher among edulis-like morphotypes than among trossulus-like phenotypes (Penney and Hart, 1999). These results are in good agreement with the data from the California mussel study mentioned earlier, in which the authors also noted a low introgression level with a slight predominance of its *M. trossulus* → *M. galloprovincialis* direction (Saarman and Pogson, 2015). It can be assumed that introgression asymmetry is a general rule that results from the different ages of species (differences in occurrence over time) (Kartavtsev, 2013b; Kartavtsev et al., 2018b).

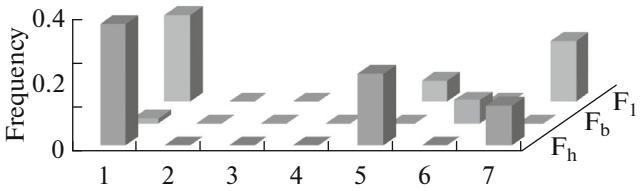

Fig. 1. An example of the frequency variation of three types of hybrids (F_h, F_b, F_1) of the mussel complex *Mytilus* ex. group *edulis* in the Sea of Japan upon the absence of gene flow between species and/or with its small effect. *X* axis: numbers of samples from mussel settlements in the Peter the Great Bay of the Sea of Japan and in close water areas; *Y* axis: frequency of hybrid specimens (according to Kartavtsev et al., 2018b).

There are numerous data indicating that mtDNA markers can penetrate from the gene pool of one species into another, where they exist for quite a long time. The penetrated foreign genes may remain in the gene pool of the species; at the same time, biological integration of the species is maintained, which was documented with the use of other MMs and phenotypic traits. Recent evidence is well supported by data for mice, frogs, fish, mussels, and other organisms (Yonekawa et al., 1981, 2000; Ferris et al., 1983; Avise, 2001; Gerber et al., 2001). Studies of mtDNA genotypes in combination with markers of nDNA or allozyme loci provided experimental evidence for the ability of mtDNA to penetrate from the genome of one species into another, bypassing species barriers, providing that hybrids between species and their offspring are viable and fertile. The given examples may indicate the absence of impairments in the nuclear-cytoplasmic balance (the regulated interaction of two genomes). This is a feature of the introgression of the mtDNA genes. The introgression of mtDNA genes occurs independently of recombination and segregation events in the nuclear genome. Thus, mtDNA components can avoid natural selection, which supports nuclear-cytoplasmic compatibility, as noted long ago (Takahata and Slatkin, 1984; Nei, 1987). Nevertheless, other facts indicating the existence of a number of selective mechanisms determining nuclear-cytoplasmic interaction have been known for a long time (Powell, 1983). In contrast to this last possibility, examples of real introgressive hybridization show that such a selection, if it exists, is not strong enough to arrest genetic introgression through mtDNA, even if the hybridization event is rare. Accordingly, cases of the detection of alien mtDNA in natural hybrids that have been identified, e.g., by MM nDNA, can be considered verified evidence of hybridization events (presumably, between closely related species/taxa). However, it is also obvious that both hybridization events and the introgression of mtDNA genes may not have a significant effect on the evolutionary fate of the involved species. Interspecific mtDNA transfer was noted for a wide range of invertebrates (*Drosophila*) and vertebrates (*Mus*, *Apodenus* and *Rana*) (Yonekawa et al., 1981, 2000; Ferris et al., 1983; Powell, 1983; Clark, 1984; Takahata and Slatkin, 1984; Spolsky and Uzzell, 1984; Nei, 1987; Avise, 2001; Suzuki et al., 2004, 2008). The literature on this topic has already been subjected to comparative analysis (Campton, 1987; Avise and Wollenberg, 1997; Yonekawa et al., 2000; Suzuki et al., 2008; Kartavtsev, 2013b, 2013c, 2015; Kartavtsev et al., 2018b). An original method of assessing the intensity of introgression was proposed (Avise, 2001); it takes into account the analysis of the nuclear-cytoplasmic equilibrium (Clark, 1984; Asmussen et al., 1987). This area is developing quite rapidly; therefore, new analytical tools appear constantly (Wiens et al., 2010; Joly, 2012). This review presents only a brief insight into mtDNA introgression in association with the potential for assessment of the status of species within BSC.

Asymmetric introgression, which is described for two frog species of the genus *Hyla*, is frequent in nature (Avise, 2001). The revealed unidirectionality was confirmed by many other studies (Kartavtsev et al., 2005, 2014, 2018b; McGuire et al., 2007; Alves et al., 2008; Plotner et al., 2008; Keck and Near, 2009; Nevado et al., 2009; Saarman and Pogson, 2015). It is the result of several biotic and abiotic factors. A similar asymmetry for certain pairs of species, which are described above and in previously published studies (Kartavtsev et al., 2005, 2014, 2018b; Kartavtsev, 2013b, 2018), leaves the gene pool of at least one of the species of the pair intact. Often, self-penetration of the mtDNA markers, plastid genes, and, obviously, some mobile elements from foreign genomes through species barriers may not lead to disintegration of the genome of individuals of the species and may not cause any direct negative effect. However, a subsequent indirect effect on the host genome is possible, e.g., through the pleiotropic action of the invading genes or genetic elements. As suggested according to BSC, new mtDNA genes and other inherited elements captured during introgression in some cases may take part in the formation of reproductive isolating barriers. The occurrence of reproductive isolating barriers

depends on the formation of further nuclear-cytoplasmic relations and on other biological and climatic events, which is probably realized now in the *Mytilus* ex. group *edulis* complex of mussel species. An example of the current development of reproductive-isolating barriers in the presence of hybridization, urbanization, global marine traiding and climate change is the various pairs of species of the described above mussel complex (Skibinski et al., 1983; Gardner and Skibinski, 1988; Rawson et al., 1996, 1999; Skurikhina et al., 2001; Kartavtsev et al., 2005, 2014, 2018b; Vainola, Strelkov, 2011; Saarman and Pogson, 2015; Katolikova et al., 2016).

The monitoring of hybridization and genetic introgression can be considered one of the most important tasks of evolutionary genetics, general genetics, and general biology. This becomes especially evident in the study of numerous examples of genetic introgression, as well as reticulatory changes in the phylogeny of marine organisms (Avise, 2001; Arnold and Fogarty, 2009; Balakirev et al., 2012, 2013). The analysis of some data on hybrid detection in animals (Arnold and Fogarty, 2009; Borkin and Litvinchuk, 2013) showed that many facts, in reality, do not represent interspecific introgression but refer to intraspecific introgression, introgression between the taxa of unclear taxonomic rank or between such taxa as subspecies/semispecies. Obviously, such cases cannot be regarded as contradictory to BSC (Kartavtsev, 2013b, 2018). The detailss of some studies (Balakirev et al., 2013) are obscure and, evidence in them rather, represent the case of a very rare double recombination that occurred during the artificial crossing of distant parents or, probably, during rare natural hybridization in the past. Thus, in one case, the mtDNA genes of *B. lenok* probably penetrated from a female of another species, which was revealed by the presence of sequences of two genes of *Hucho taimen* genome (Balakirev et al., 2013). However, as follows from another source, this crossing direction results in hybrid incompatibility (Wang et al., 2011), whereas the opposite crossing direction gives even heterosis (Xu et al., 2011). In the described case (Balakirev et al., 2013), there is a possibility that the detected recombination, which is interpreted as a consequence of extensive natural hybridization, is in fact a consequence of artificial crosses or laboratory manipulations with genomes. These data, in any case, do not provide any evidence for the prevalence of hybridization among the described species of salmon fishes from different families and do not prove the fact of hybridization at all, since they do not rely on diagnostic markers of nDNA. The other review (Arnold and Fogarty, 2009) provide examples of introgression; some of these examples indicate the presence of hybrids but do not prove the fact of genetic introgression. As noted earlier, the presence of F_1 hybrids does not prove the existence of gene introgression (Campton, 1987). For some fish species hybridization is common (Campton, 1987), but it only sporadically leads to actual genetic introgression (Avise and Saunders, 1984). Convincing data that hybridization events and subsequent introgression were associated with large-scale climate changes, as follows from the examples for chars of the genus *Salvelinus*, were also presented (Glemet et al., 1998; Oleinik and Skurikhina, 2010; Roberts et al., 2010; Oleinik, 2013). Such events can be directly or indirectly due to human activity (Altukhov and Salmenkova, 1991; Avise, 2001). The above review (Arnold and Fogarty, 2009) provides examples of hybrid detection by morphological features, but this should hardly be taken as evidence not only of genetic introgression but even the presence of hybrids (Campton, 1987; Grant, P.R. and Grant, R.B., 1992).

Detailed studies of fish and mussel species based on mtDNA and nDNA markers revealed that most of the identified hybrids were F_1 descendants, which can be considered a weakness or complete absence of real genetic introgression (Avise and Sunders, 1984; Kartavtsev et al., 2005, 2014, 2018b; Oleinik and Skurikhina, 2010; Saarman and Pogson, 2015). However, there are studies that reliably proved the mass character of advanced fish hybrids in F_2, F_3, etc. (Roberts et al., 2010; Nevado et al., 2011). In one case, mtDNA markers and nuclear microsatellite loci showed the presence of descendants of backcrosses of F_1 specimens with one of the parental species with a high frequency of 44.9% (Roberts et al., 2010). In another case, with different combinations of nDNA and mtDNA markers, gene flow was noted between four cichlid species of the genus *Ophthalmotilapia* from Lake Tanganyika (Nevado et al., 2011). The authors of the cited study reported a significant proportion of specimens of the ubiquitous species *O. nasuta* in the lake, which had partly mtDNA and nDNA markers typical of the other three nominal species of the genus. All such specimens were found in populations sympatrically living with other species of the genus. The authors suggest that this feature appeared as a result of sequential and independent episodes of genetic exchange in different parts of the lake with unidirectional introgression into *O. nasuta* genome. From the presented materials, it follows that, even in cases of careful analysis, accurate estimates of the proportions of F_2 and similar descendants, which is required for the assessment of genetic introgression, are not always available. Regarding the second study cited above, there is also the issue of the correspondence of the studied taxa to the species status in agreement with BSC, although the authors consider the genus *Ophthalmotilapia* as an old, valid branch of taxonomic species (Nevado et al. 2011). However, there are opposite opinions about cichlids (Mayer et al., 1998; Turner et al., 2001). Another addition to this criticism is the fact that, in some studies, the differentiation of offspring by F_1, F_2, F_3, etc., categories is absent. For example, the aforementioned review (Arnold and Fogarty,

2009) of genetic data on introgression for 27 outbred species from 32 compared species and other categories are shown in summary in Table 1. The vast majority of data should indicate, according to the authors, the existence of genetic introgression. However, if we analyze this information in accordance with the requirements presented earlier, then only a small fraction of the entire data set remains acceptable as evidence of genetic introgression, while the rest relate to exchanges at the intraspecific level between subspecies or taxa of unclear status.

The presented introgression cases, e.g., for marine flora, algae, and other vegetation (Balakirev et al., 2012), were generally consistent with the known data for terrestrial plants (Schneer and Rodionov, 2018); i.e., these data confirmed the weakness of the reproductive barriers in many plant species. In any case, one should be careful when declaring the detection of hybrids by mtDNA markers: the identification of hybrids according to the variability of mtDNA can lead to false conclusions. For example, mussels have a double uniparental inheritance of mtDNA. In their evolutionary history, the nucleotide sequences of the 16S rDNA of the maternal and paternal lines diverged by 8.3% within the same species (Rawson and Hilbish, 1995). Accordingly, analysis of species divergence without allowance for gender differences can lead to erroneous conclusions.

Additional details from theoretical and empirical studies of the occurrence of hybrids, hybridization, and genetic introgression can be found (Borkin and Litvinchuk, 2013; Barton and Hewitt, 1985; Smith, 1992; Arnold, 1997, 2008; Hewitt, 2011; Saarman and Pogson, 2015). Based on the data described above, it is clear that many aspects of hybridization are quite complex and not completely clear; thus, quick and clear answers to all questions cannot be expected. Among vertebrates, birds are more often mentioned as examples of the occurrence of hybridization 10–19% (Panov, 1989; Grant, P.R. and Grant, R.B., 1992; Aliabadian, Nyman, 2007), while amphibians and fish are cited less frequently in this respect, though more often than reptiles and mammals (Borkin and Litvinchuk, 2013). Although there are no clear statistics on this topic, this impression may be due to the poor representativeness of the taxon sample in studies on this issue. The material presented above provides some examples of quantitative assessment of the genetic introgression level. Analysis of the data in the summary table (Arnold and Fogarty, 2009) shows that the actual number of cases of genetic introgression is overestimated. As we mentioned earlier, the frequency of introgressive hybridization in animals is estimated at 10% (Arnold, 1997); therefore, the proportion of genetic introgression in reality should be lower. In any case, to support the objective of the present study, it can be concluded that, regardless of compliance or noncompliance with BSC, species existing in nature as biological entities are mainly capable of maintaining their integrity and independence; at least, this idea can be traced in the studied retrospective and the prospective outlook. The following data should help in our understanding of the association of taxonomy, and more broadly, BSC/STE, with molecular evolution.

PREDOMINANT TOPOLOGY OF GENE TREES, GENETIC DIVERGENCE LEVELS BETWEEN TAXONS, AND DEGREE OF CONSENT OF MOLECULAR DATA WITH BSC/STE AND DNA BARCODING PRACTICE

Clarification of the Species Concept

The study tasks require clarification of the essence of the discussed species. According to one of the first definitions in the BSC framework, a species is considered as the reproductive community of populations (isolated from other populations) that occupy a specific niche in nature (Mayr, 1982, p. 273). Such a concept of species in the BSC framework is accepted in the study as the basis of the stated facts and ideas, although it is limited mainly to bisexual organisms and has several clarifications (Mayr, 1968; Timofeev-Resovky et al., 1977; Templeton, 1998; Kartavtsev, 2005).

In general, at least seven different definitions of a species can be listed (Mayr, 1982; Simpson, 1961; Paterson, 1978, 1985; Wiley, 1978; Crawcraft, 1983; van Vallen, 1976; Templeton, 1998). The well-known idea (Dobzhansky, 1955) allowed justification (Bush, 1975) of the key role of the process of termination of the gene flow; this separates the original species into two or more reproductively isolated units, leading to speciation. It can even be stated that, if there is undeniable evidence that the appearance of the species and its evolution are possible without termination of the gene flow between the ancestral forms and that the species can arise in the presence of wide genetic exchange, then, of course, it will be necessary to reject the BSC/STE concept. Similarly, if a speciation model exists and prevails in nature, according to which a new species can form without long-term separation of the gene pools of the parent and daughter forms and without the emergence of reproductive isolating barriers, then the paradigms of BSC/STE should be replaced. Accepting BSC/STE concept, one should also recognize the direct link between genetic distance and time, which was clearly stated for protein loci (Nei, 1987) and later extended for any MM (Drummond and Bouckaert, 2015).

Empirical Information on the Topology of Gene Trees, Levels of Genetic Divergence between Taxa

There are different types of gene trees, but most of them look like monophyletic or sometimes paraphyletic (polyphyletic) dendrograms. Direct assessments of the congruence of different gene trees or the

expected topology, for example, of the species tree, are not frequent, and, when performed, they show results ranging from good to excellent (Birky, 2013; Hedges et al., 2015; Turanov et al., 2016; Kartavtsev et al., 2017). An unsatisfactory "convergence" of the topology of trees of different genes can also be observed, but this is more often due to normal lineage sorting for different genes and to the informational and technical laboratory capabilities of the reconstruction, e.g., an insufficient information capacity of the used sequences (small length) with a large number of analyzed operational taxonomic units (OTU) or the inappropriate selection of MMs for the identification of the phylogenetic signal during tree reconstruction and other operational errors. Due to the random sorting of phyletic lineages by individual genes, the use of mitogenomes may be a more appropriate approach. It should be also clarified that some groups intended for analysis in numerical systematics were historically called the operational (operable) taxonomic unit (Sneath and Sokal, 1963). However, in reality they represent certain populations of organisms, certain taxonomic groups such as species and genus, that have a set of similar members or branches (Bailey, 1967, p. 156). Usually, the semantic understanding of OTU is given for a species rank study.

The success of phylogenetic-tree construction on the basis of a complete mitogenome has been demonstrated for 100 fish taxa (Miya et al., 2003), as well as for many other groups and analysis options, including 13 protein genes of the mitogenome of Pleuronectiformes (Kartavtsev et al., 2016) or carp fish Cypriniformes, Cyprinidae (Imoto et al., 2013; Kartavtsev et al., 2017) and for many other fish taxa; representative samples of their nDNA markers were also included in the analysis (Berendzen and Dimmick, 2002; Pardo et al., 2003, 2005; Saitoh et al., 2006; Mayden et al., 2009; Betancur-R and Orti, 2014). A complex approach with numerical simulation and the use of temporal trees for a large number of selected eukaryotic nDNA candidate genes from 2274 studies representing 50 632 species (sequence samples) of the global tree of life, found that genetic divergence increases with increasing taxon rank (Hedges et al., 2015). The main conclusions from the presented materials and the predominant topological signal of gene trees are as follows: (1) in most trees, there are obvious branches of the outer group; (2) the main branches (nodes, clusters) are represented by families/subfamilies within the taxa of the order rank, (3) there are distinct branches representing different genera below in the hierarchy; and (4) there are sets of the closest branches, which consist of specimens that are classified as specimens of the same species. Some of the trees contain obscure intrageneric and intrafamily clusters, which in most cases represent paraphyly or polyphyly within a taxon, which require explanation (usually, the latest data leads to taxon revision in systematics).

A difficult question is how to deal with this whole array of information? For example, there is currently no general approach to the estimation of the number of erroneous assignments to a single cluster or the errors of neighbor identification by OTU in gene trees selected from studies and presented in the literature. Accordingly, there are no obvious ways to assess the degree of reticulation or polytomy inside trees. Attempts to find a common solution for quantitative evaluation of biodiversity were made based on several methods within the framework of DNA barcoding tools (Bringloe et al., 2016; Kartavtsev, 2018; Stoeckle and Thaler, 2018), although each of the cited studies was focuses on different goals. The authors are aware of four general approaches to this problem: (1) Poisson tree processes (PTP) (Zhang et al., 2013); (2) a method similar to PTP, or a coalescence approach according to Generalized Mixed Yule Coalescent (GMYC) theory (Fujisawa and Barraclough, 2013); (3) a comparison of bifurcations in species trees reconstructed from sequences (Joly, 2012); and (4) a GMYC-like ideology, the K/θ approach (Birky, 2013).

For example, there are complications in the interpretation of data of *Co-1* and *Cyt-b* gene trees of flounders, which contain intrageneric paraphyletic clusters for the genera *Hippoglossoides* and *Pseudopleuronectes* (Kartavtsev et al., 2016, Figs. 1–2). Although for these two genera, this fact simply reflects the incorrect morphological identification of some specimens; in other words, the given example illustrates the synonymy of names for the same species that have already been discussed (Kartavtsev et al., 2007, 2016). This is a question that can be successfully resolved with DNA barcodes. Other DNA barcoding problems are also possible. Such problems were detected in the study of earthworms, among which cryptic biodiversity was detected by DNA barcodes but without a clear taxonomic signal. Various authors have also raised the question in a broader context with the diagnostics of living forms in taxa poorly developed by taxonomists (Stoeckle and Thaler, 2018). More often, however, the problem of identification is solved by a more careful selection of MMs via the production of a reference library of barcodes for a particular taxon or problem in question and clarification of the understanding of the species in the taxon. Misclassifications found by MMs within OTU exacerbated a problem that was known in taxonomy for specialists, i.e., the existence of numerous synonymous names (Bringloe et al., 2016; Bayne et al., 2017; Kartavtsev, 2018), which usually provokes multiple taxonomic revisions. Another obvious problem arises from the inability to distinguish sometimes between taxonomic error in specimen identification and false clustering by MMs in the gene or species tree caused by genetic reticulation. It is especially difficult to solve this task for taxa that are far from the direct expertise of the authors. There are other complications, e.g., inconsistency in the evolution rates and, as a result, the mismatch of trees derived based on

mtDNA and nDNA markers (Wiens et al., 2010), differences in the substitution (mutation) rates for different genes, the sorting of phylogenetic lines associated with a genetically effective size of founding populations, and some others (Nei, 1987; Avise, 2000; Drummond and Bouckaert, 2015).

This study proposes an approach to the indicated problem based on MMs and BOLD (Kartavtsev, 2018). The following question has been posed: is there consistency between (1) molecular classifications according to DNA barcoding data expressed in the values of BIN metric and (2) specimens classified by experts in systematics and also placed in BOLD (Kartavtsev, 2018). As we noted earlier, the BIN is the assignment of a unique identifier (or a special index) to each specimen, which makes it possible to assign a newly tested specimen to a particular taxon from BOLD. BIN values (=OTU) in BOLD are now determined mainly based on *Co-1* sequences. The base is replenished quickly with other markers. BIN values are independent of the previous taxonomic identifications of specimens; e.g., there are two rows of independent variables suitable for comparison. In other words, with the use of BIN, it is possible to assess the correspondence between clusters of DNA barcodes and OTU descriptions of the same specimens in the database, which are designated within the framework of traditional systematics. The BIN values can reveal the agreement of two independent classifications or an inconsistency of classifications in matched arrays of samples in BOLD, which forms the basis for a statistical assessment of the degree of similarity of these two data scores. Since the obtained signal is related to the hierarchical tree-like reconstructions, we can speak of their congruence.

The cited paper (Kartavtsev, 2018) analyzed data from BOLD from three projects: TZFPC (Steinke et al., 2009), FERU (Turanov et al., 2014), and SCFAA (McCusker et al., 2013). Taxonomic expertise is provided by the authors of the submitted projects. These data regarding fish were chosen for simplicity of work as the closest to the professional interests of the authors of the study. The results of the analysis with BIN values revealed that 81.4 ± 2.3% of specimens of species, 84.0 ± 3.9% of specimens of genera (Fig. 2), and 88.0 ± 5.8% of specimens of families (Kartavtsev, 2018) of these BOLD projects were consistent with the preliminary zoological classifications of presented fish taxa. It turned out that, even at the family level, a single *Co-1* MM with an incomplete nucleotide sequence is well suited for identifying specimens. It also turned out that there are no significant differences between the three taxonomic levels (Kartavtsev, 2018, Table 1S), and the data from two different projects are not statistically significant (Fig. 2). Additional details of this analysis can be found in the cited paper and the attached electronic materials (Kartavtsev, 2018, Table 1S). In the presented analysis, the log-transformed numbers of all BIN values (specimens determined morphologically) and concordant or consistent classifications among BIN values (specimens identified by MM) showed proportional-positive variability, which was well approximated by a linear regression equation; The analysis results are valid for combined data from our own research and another source, data from FERU/TZFPC, and literature data on the SCFAA project. The coefficient of determination of this linear dependence was about 90% ($r_p = 0.989$, $P < 0.001$, for the least effect; Kartavtsev, 2018). The information presented in Fig. 2 and these data imply that all sequences/specimens of the same species are determined in the majority of cases to be clusters of the same species, whereas different species are classified into separate genera, in accordance with traditional taxonomic practice. The same conclusion holds for clustering specimens of different genera. These two types of branches at the intra- and interspecific levels are clearly distinct based on any of the genetic distances scales (Kartavtsev and Lee, 2005; Kartavtsev, 2009) and used as one of the main means of DNA barcoding in iBOL and similar projects. Based on the above material, it is now clear why there is an obvious general property of the formation of MM clusters, including DNA barcodes by *Co-1*, which represent a biological species. There are exceptions to the general rule when the species is represented by a single reproductive unit. Exceptions include cases with phylogeographic subdivision of species due to the degree of subspecies isolation (Avise, 2000). There are also situations in which species have common or overlapping clusters of DNA barcodes due to the complex history of their mtDNA formation and introgression (Stoeckle and Thaler, 2018, Figs. 2 and 3), which complicates the identification task. There may be exceptions associated with biology that lead to the absence of MM divergence, e.g., when speciation is not associated with the geographical D1 model (according to the classification of Templeton, 1981) with an accumulation of mutation-substitutions during long-term isolation but is due to regulatory gene changes and chromosomal transformations: the D3 and T2–T4 speciation methods (Templeton, 1981).

Prior to the subsequent analysis, in which genetic-distance data calculated based on MM sequences will be considered, let us turn once again to phylogeny at the family level. This is a very important point. The presence of monophyly at this level is of fundamental importance, especially for the members-rich families like flatfish, Pleuronectidae, Soleidae, and Bothidae, for which the basic information is morphological–anatomical (FishBase; www.fishbase.org). Fortunately, comparative information for these taxa has also been obtained based on molecular phylogenetics (Berendzen and Dimmick, 2002; Pardo et al., 2005; Sharina and Kartavtsev, 2010; Betancur-R and Orti, 2014). Monophyly of at least three phyletic lines (families) within Pleuronectoidei was originally based on *12S* and *16S* rDNA data (Berendzen and Dimmick,

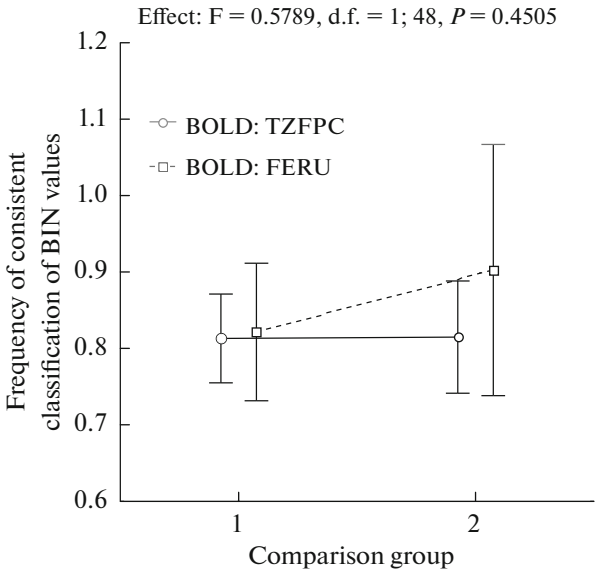

Fig. 2. Two-factor ANOVA of the distribution of average BIN values in two fish studies summarized by BOLD. The mean values for BIN show consistent classifications (along the *Y* axis, *%*) for the *Co-1* MM of species/specimens mtDNA and its consistency with the independent diagnosis based on the traditional traits presented in BOLD. Comparison groups (*X* axis) are presented in two categories: individual species (1) and independent genera (2). The values of the consistent classifications do not differ in the two compared groups (1 and 2). They also do not differ between the estimates in the two studies (Steinke et al., 2009, TZFPC; Turanov et al., 2014, FERU), which were represented by 1219 and 285, as well as 199 and 64, analyzed samples and species, respectively. The overall consistency of classifications based on BIN estimates was more than 82% (points with confidence intervals on the graph); therefore, the value of erroneous classifications is less than 18% (according to Kartavtsev, 2018).

2002) and *16S* rDNA for several families (Pardo et al., 2005). It was later supplemented with data for *Co-1*, *Cyt-b*, and mitogenome, mainly for Pleuronectidae (Kartavtsev et al., 2016). A complete understanding of phylogeny for the latter family is in development and recently was supplemented with MMs for several mtDNA and nDNA genes (Betancur-R and Orti, 2014; Vinnikov et al., 2018). Moreover, according to some data, monophyly looks quite convincing for the whole order (Betancur-R and Orti, 2014; Kartavtsev et al., 2016). Some complications, such as the presence of paraphyly in the Pleuronectinae subfamily and in the genus *Limanda*, which were recently established based on of larval morphology and molecular phylogenetic data, are obviously inevitable (Roje, 2010; Kartavtsev et al., 2016). There is a report on the interspecific hybridization of some taxa of European flounders (Kijewska et al., 2009). However, it is not yet clear whether paraphyly exist for these taxa or whether polytomy should be attributed to the inadequacy of morphological characters for identification, and not to hybridization and the formation of fully hybrid populations (and potential taxa) in the area of the last study in the Baltic Sea but also in the neighboring basins of Europe. However, such situations are not excluded here, as noted, e.g., for mussels in this region (Smietanka et al., 2014; Väinölä and Strelkov, 2011).

Another taxon, the carp-like Cypriniformes, was subjected to genetic, biochemical and molecular phylogenetic studies even more intensively than flounders (Hanzaman et al., 1984; Kartavtsev et al., 2002; Miya et al., 2006; Sakai et al., 2006; Semina et al., 2006; Saitoh et al., 2006, 2010; Sasaki et al., 2007; Mayden et al., 2009; Batischeva et al., 2011; Imoto et al., 2013; Kartavtsev et al., 2017). For these fishes, despite numerous hybrids, the existence of polyploid forms and the assumption of speciation through hybridization (Saitoh et al., 2010; Yang et al., 2015), as well as the available data for trees of individual genes and mitogenomes, indicate that reticular evolution is not predominant. Trees are usually of the bifurcation type and have monophyly in the overwhelming majority of taxa branches. Of course, there are certain problems in the molecular systematics of this very diverse taxon. They will continue to arise during the construction of large trees and in their comparison with small trees. For example, it was found that the large-gene trees for Leuciscinae have a much smaller congruence as compared to smaller trees based on the software package Dendroscope (Kartavtsev et al., 2017). However, it is looks like that this only requires an increase in the information capacity of the signal for better resolution of the topology.

In addition, there is information that contradicts the above data supporting the presence of reticulations in the tree topology. A polytomy signal was found in trees constructed for taxa from hybrid zones, as was recently documented for *Mytilus* ex. group *edulis* complex (Smietanka et al., 2014; Zbawicka et al., 2014, 2018). Complexes with a rich variety of tropical and neotropic fauna are also examples of reticulations (Nevado et al., 2011; Pereira et al., 2013). In the already cited review (Arnold and Fogarty, 2009, Table 1) there are 17 findings of phylogenetic mismatches. However, these facts do not disprove the main signal, which indicates the prevalence of bifurcations and monophyly in gene trees, as well as the ability of DNA and other MMs to identify fish taxa with an accuracy higher than 80% (Pereira et al., 2013; Turanov et al., 2014; Kartavtsev, 2018). Evidence based on DNA barcoding and extensive BOLD materials supports these conclusions for hierarchical categories up to the generic level (the main focus of the iBOL program). However, the facts above unexpectedly prove the spread of this signal to the family level. A good correspondence of the topological signal of gene trees according to DNA barcode data (e.g. for shallow phylogenies) and existing taxonomic hierarchies was recently demonstrated for several phyla, including Chordata, Arthropoda, Mollusca, and Echinoder-

mata, which represent 75% of all described species of these taxa (Stoeckle and Thaler, 2018).

As previously assumed, we will consider data on genetic distances and their correspondence to the ranks of evolutionary divergence. One set of such data was summarized for two mtDNA *Co-1* and *Cyt-b* genes (Kartavtsev 2009b, 2011a, 2011b, 2013a, 2015, Ch. 7). These data will be used for the conclusions given below. This review presents new data showing the variability of mtDNA for the *16S* rRNA gene and the complete animal mitogenome in the hierarchy of taxa or comparison groups (Figs. 3a and 3b). The *16S* rRNA sequences of various animal species with a length of 191–444 bp after alignment were extracted from the GenBank at May 1, 2018 and aligned with the MEGA-6 software package (Tamura et al., 2013). Then, using the same software package, the authors calculated the pairwise *p*-distances for two samples of 6 673 and 693 specimens, respectively, for the first and second half of the sequences of the entire initial set (this procedure was required, since it was impossible to perform the alignment at the same time due to large differences between the sequences in the initial GenBank file). The variability within and between the groups was then compared with one-way analysis of variance (ANOVA) in the software package Statistica-6 (STATISTICA, 2001) (Fig. 3a). An analysis of 7459 sequences of the complete mitogenomes of animals with an average length of 15173 bp (GenBank data, February 13, 2006–April 27, 2018, length: 15667, 15347, 14761, 16117, 13974 bp) was performed approximately in the same order. However, due to the large data arrays, calculations of the *p*-distances were initially performed separately for each of the comparison groups and then summarized in a general table for all groups. The table was used for ANOVA performed with Statistica-6 (Fig. 3b). A nonparametric Kruskal–Wallis variance analysis was also performed with consideration of deviations from the normal distribution of the variation rows of *p*-distances (although for presented files they were unimodal and suitable for this analysis), complementary to the parametric ANOVA shown in Fig. 3. This testing confirmed the significance of the difference between the compared groups, both for the *16S* rRNA gene sequences and for the mitogenome sequences. For clarification, the calculation details can be provided by the authors to interested readers upon request. The data on genetic distances within species and in the hierarchy of taxa convince us that there is a relationship in most of the studied phyla between the *p*-distances and taxon rank that is close to linear, with minimal values in the comparison group within the species. As follows from the obtained data, species clusters on a vast array of DNA barcode data are clearly distinguished not only by individual genes but also by mitogenomes in general (Fig. 3b) (Stoeckle and Thaler, 2018). There are also exceptions to this rule when gaps between species clusters are minimal or absent altogether (Turanov et al., 2014; Kartavtsev et al., 2017; Stoeckle and Thaler, 2018). Basically, such cases are related to taxa that have not reached the species level (subspecies, semispecies, or young species), but they may also be due to the presence of species (forms) that do not form reproductive units with sexual reproduction, e.g. species that do not fall within the BSC. These species may occur as a result of genetic transformation that does not affect or has little effect on structural genes (Kartavtsev, 2009a, 2009b, 2011a, 2011b, 2013a), as noted above. There are also examples of significant variability of the molecular evolution rate for taxa of the same rank in various phyla, which may complicate the use of genetic distances for interspecific comparisons (Avise and Aquadro, 1982; Avise, 2000; Kartavtsev, 2009a, 2009b, 2011a, 2011b, 2013a, 2013c). Therefore, the universality of the DNA barcode and genetic-distance scale is not absolute, which is not fully understood in some generalizations on DNA barcoding (Stoeckle and Thaler, 2018). The solution is the creation of algorithmic tools and software of a more universal approach that takes into account the need to use different measures of genetic divergence and variability with the use of several genotypic descriptors, as well as phenotypic identifiers (Kartavtsev, 2009a, 2011a, 2011b, 2013a, 2015).

Summarizing all of the obtained data, we can formulate three main conclusions.

Conclusion 1. The delimiting of a species is highly efficient due to the low intraspecific and significant interspecific variability of the used MMs (Fig. 3, groups 1 and 2), which is detected both at the level of individual markers of mtDNA (*Co-1*, *Cyt-b*) and *16S* rDNA and at the level of complete mitogenomes. This conclusion applies to a wide range of animal taxa, for which there is a certain conceptual clarity of what a species is.

Conclusion 2. The positive relationship between the distance and rank of the taxon with the minimum at the intraspecific level proves that speciation mainly occurs in accordance with the D1 geographical model (Templeton, 1981); in the animal world, phyletic evolution prevails. The latter assertions fully confirm the basic principles of BSC and, in general, the neo-Darwinian interpretation of speciation and evolution.

Conclusion 3. From the presented materials, it follows that alternative models of speciation (D3, T2–T4, etc.) are rare in nature. In the opposite case, e.g. if a variety of equally probable methods of speciation are implemented in nature, the distance distribution, as ranked by taxa, would have a uniform appearance or a slight slope. It is obvious that the presented analysis and other evidence refute this possibility. However, this does not mean that there are no alternative mods of speciation or that they are less important. The validity of the well-known proposition that Darwinian evolution, which results in the observed diversity of living forms, prevails over time is confirmed, but fundamental genetic transformations, which rarely occurring in

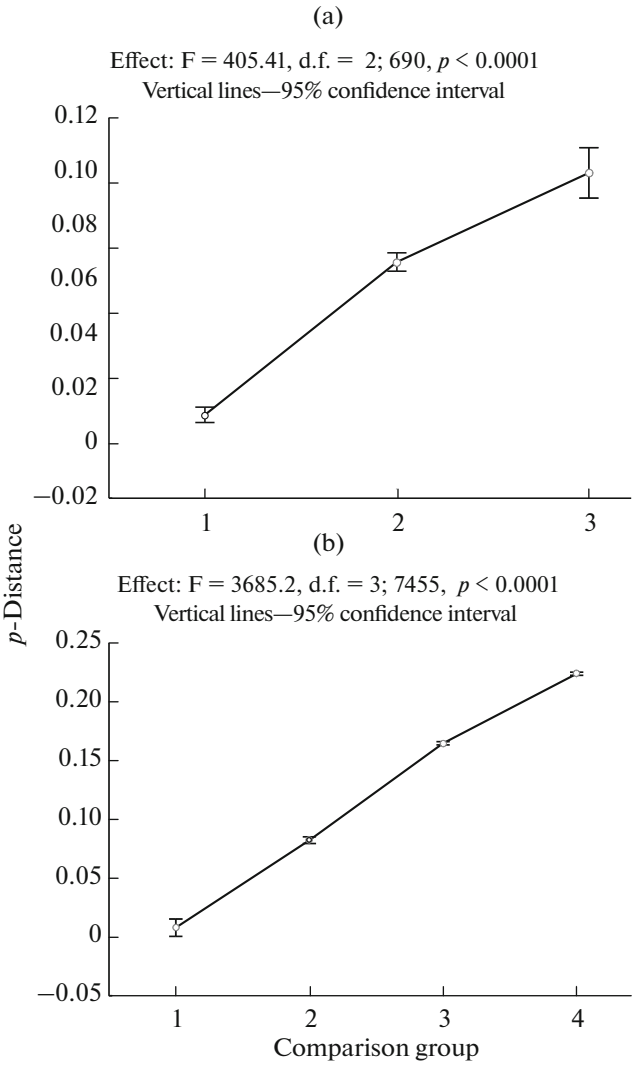

Fig. 3. Univariate ANOVA showing the variability of the mean values of *p*-distance (*Y* axis) for the comparison groups of the sequences of *16S* rRNA and animal mitogenomes. Comparison groups (*X* axis): 1—within species; 2—within the genera; 3—within subfamilies-families; 4—within the order; (a) data from sequence analysis of *16S* rRNA of animals of the second set of sequences (GenBank, May 5, 2018); (b) data on the analysis of the sequences of the complete mitogenome of animals (GenBank, February 13, 2006—April 27, 2018).

the course of evolution, lead to aromorphoses and lead to fundamental innovations of living matter. However, this is a controversial issue that has not yet found a reliable factual justification.

CONCLUSIONS

Species delimitation based on molecular markers, or, more precisely, based on DNA nucleotide sequences or DNA barcodes, is very successful, as evidenced by the extensive data from the global database BOLD. However, a theory that would explain this fact has not yet been created. In the present study, an approach based on the value of the DNA barcoding index, BIN, is proposed. Using the BIN and the BOLD array, we managed to identify (distinguish) taxa of three ranks (species, genus and family) and make a statistical assessment of identification consistency, reaching more than 80%. In addition, it has been proven for the majority of gene trees constructed for the *Co-1* marker that there is a good agreement between their topology and the generally accepted taxonomic classification in the taxon hierarchy (species, genus, family), at least for the analyzed fish data. The knowledge gained on deviations from the predicted values for species identification and the determination of the rank of other taxa will improve the system for the description of biodiversity on an accurate molecular and bioinformational basis. The main conclusion from the analysis is that successful identification by molecular markers arises due to the prevalence in nature of the geographical mode of speciation. During the long-term process of species formation with the accumulation of mutations and the corresponding numerous nucleotide substitutions in various daughter ancestral taxa, unique nucleotide sequences and DNA barcodes, which are detected by molecular markers, can occur. The subsequent independent evolution of the arising phyletic lines, including further divergence to the level of the genus, family, and further, constitutes the actual basis of the detected relationship of the BIN value and rank for the three taxa identified by the traditional morphological approach. In general, the relationship between genetic distances and taxon rank was proven for various groups of animals according to data for individual genes and for complete mitogenomes.

FUNDING

The study was supported by a grant of the Russian Science Foundation (Agreement no. 14-50-00034) in the field of molecular systematics of marine organisms, as well as a grant of the Russian Foundation for Basic Research 15-29-02456-ofi for research on the genetic basis of biodiversity, and a grant of the Far East Branch of the Russian Academy of Sciences, no. 18-4-040 for the Comprehensive Study of the Biodiversity of Fishes and Invertebrates Based on DNA Barcoding, Development, and Support of Databases and Biobanking.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

Aliabadian, M. and Nijman, V., Avian hybrids: incidence and geographic distribution of hybridization in birds, *Contrib. Zool.*, 2007, vol. 76, no. 1, pp. 59–61.

Altukhov, Yu.P. and Salmenkova, E.A., The genetic structure of salmon populations, *Aquaculture,* 1991, vol. 98, pp. 11–40.

Altukhov, Yu.P., Salmenkova, E.A., and Omel'chenko, V.T., *Populyatsionnaya genetika lososevykh ryb* (Population Genetics of Salmon Fishes), Moscow: Nauka, 1997.

Alves, P.C., Melo-Ferreira, J., Freitas, H., and Boursot, P., The ubiquitous mountain hare mitochondria: multiple introgressive hybridization in hares, genus *Lepus, Philos. Trans. R. Soc., B,* 2008, vol. 363, pp. 2831–2839.

Antonov, A.S. and Belozerskii, A.N., Comparative analysis of nucleotide composition of deoxyribonucleic acids of some vertebrates and invertebrates, *Dokl. Akad. Nauk SSSR,* 1961, vol. 138, pp. 1216–1220.

Antonov, A.S., Miroshnichenko, G.P., and Slyusarenko, A.G., The data on DNA primary structure in the plant systematics, *Usp. Sovrem. Biol.,* 1971, vol. 74, pp. 247–261.

Arnold, M.L., *Evolution through Genetic Exchange,* New York: Oxford Univ. Press, 1997.

Arnold, M.L., *Evolution through Genetic Exchange,* New York: Oxford Univ. Press, 2008.

Arnold, M.L. and Emms, S.K., Paradigm lost: natural hybridization and evolutionary innovation, in *Endless Forms: Species and Speciation,* Howard, D.J. and Berlocher, S.H., Eds., New York: Oxford Univ. Press, 1998, pp. 379–389.

Arnold, M.L. and Fogarty, N.D., Reticulate evolution and marine organisms: the final frontier? *Int. J. Mol. Sci.*, 2009, vol. 10, pp. 3836–3860. https://doi.org/10.3390/ijms10093836

Asmussen, M.A., Arnold, J., and Avise, J.S., Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes, *Genetics*, 1987, vol. 115, pp. 755–768.

Avise, J.C., *Phylogeography. The History and Formation of Species,* Cambridge, MA: Harvard Univ. Press, 2000.

Avise, J.C., Cytonuclear genetic signatures of hybridization phenomena: rationale utility and empirical examples from fishes and other aquatic animals, *Rev. Fish Biol. Fish.,* 2001, vol. 10, pp. 253–263.

Avise, J.C. and Aquadro, C.F., A comparative summary of genetic distances in the vertebrates: pattern and correlations, *Evol. Biol.,* 1982, vol. 15, pp. 151–185.

Avise, J. and Saunders, N.C., Hybridization and introgression among species of sunfish (*Lepomis*): analysis by mitochondrial DNA and allozyme markers, *Genetics,* 1984, vol. 108, pp. 237–250.

Avise, J.C. and Wollenberg, K., Phylogenetics and origin of species, *Proc. Natl. Acad. Sci. U.S.A.,* 1997, vol. 94, pp. 7748–7755.

Bailey, N.T.J., *The Mathematical Approach to Biology and Medicine,* London: Wiley, 1967.

Balakirev, E.S., Krupnova, T.N., and Ayala, F.J., DNA variation in the phenotypically-diverse brow alga *Sacharina japonica, BMC Plant Biol.,* 2012, vol. 12, pp. 108–126.

Balakirev, E.S., Romanov, N.S., Mikheev, P.B., and Ayala, F.J., Mitochondrial DNA variation and introgression in Siberian taimen *Hucho taimen, PLoS One,* 2013, vol. 8, no. 8, p. e71147.

Barley, D.M., Rana, K., and Immink, A.J., The use of inter-specific hybrids in aquaculture and fisheries, *Rev. Fish Biol. Fish.,* 2001, vol. 10, pp. 325–337.

Barton, N.H. and Hewitt, G.M., Analysis of hybrid zones, *Ann. Rev. Ecol. Syst.,* 1985, vol. 16, pp. 113–148.

Bayne, B.L., Ahrens, M., Allen, S.K., et al., The proposed dropping of the genus *Crassostrea* for all Pacific cupped oysters and its replacement by a new genus *Magallana*: a dissenting view, *J. Shellfish Res.,* 2017, vol. 36, no. 3, pp. 545–547.

Berendzen, P.B. and Dimmick, W.W., Phylogenetic relationships of Pleuronectiformes based on molecular evidence, *Copeia*, 2002, vol. 3, pp. 642–652.

Betancur, R.R. and Orti, G., Molecular evidence for the monophyly of flatfishes (Carangimorpharia, Pleuronectiformes), *Mol. Phyl. Evol.,* 2014, vol. 73, pp. 18–22.

Birky, C.W., Jr., Species detection and identification in sexual organisms using population genetic theory and DNA sequences, *PLoS One,* 2013, vol. 8, no. 1, p. e52544. https://doi.org/10.1371/journal.pone.0052544

Borkin, L.Ya. and Litvinchuk, S.N., Hybridization, speciation, and systematics of the animals, *Tr. Zool. Inst., Ross. Akad. Nauk*, 2013, suppl. 2, pp. 83–139.

Bringloe, T.T., Cottenie, K., Martin, G.K., and Adamowicz, S.J., The importance of taxonomic resolution for additive beta diversity as revealed through DNA barcoding, *Genome,* 2016, vol. 59, no. 12, pp. 1130–1140.

Brower, A.V.Z., Delimitation of phylogenetic species with DNA sequences: a critique of Davis and Nixon's population aggregation analysis, *Syst. Biol.,* 1999, vol. 48, pp. 199–213.

Busack, C.A., Torgaard, J.H., Bannon, M.P., and Gall, G.A.E., An electrophoretic karyotypic and meristic characterization of the Eagle Lake trout, *Salmo gairdnery aquilarum, Copeia,* 1980, vol. 3, pp. 418–424.

Bush, G.L., Modes of animal speciation, *Ann. Rev. Ecol. Syst.,* 1975, vol. 6, pp. 339–364.

Campton, D.E., Natural hybridization and introgression in fishes. Method of detection and genetic interpretation, in *Population Genetics & Fishery Management,* Ryman, N. and Utter, F., Eds., Seattle: Washington Sea Grant Program, 1987, pp. 161–192.

Clark, A.G., Natural selection with nuclear and cytoplasmic transmission. I. A deterministic model, *Genetics*, 1984, vol. 107, pp. 679–701.

Delaney, M.E. and Bloom, S.E., Replication banding patterns in the chromosomes of rainbow trout, *J. Hered.*, 1984, vol. 5, pp. 431–434.

Devlin, R.H. and Nagahama, Y., Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences, *Aquaculture,* 2002, vol. 208, pp. 191–364.

Dobzhansky, Th., *Evolution, Genetics, and Man*, New York: Wiley, 1955.

Drummond, A.K. and Bouckaert, R.R., *Bayesian Evolutionary Analysis with BEAST,* Cambridge: Cambridge Univ. Press, 2015.

Ferris, S.D., Sage, R.D., Huang, C.-M., et al., Flow of mitochondrial DNA across a species boundary, *Proc. Natl. Acad. Sci. U.S.A.,* 1983, vol. 80, pp. 2290–2294.

Fitzpatrick, B.M., Rates of evolution of hybrid unviability in birds and mammals, *Evolution,* 2004, vol. 58, pp. 1865–1870.

Fong, J.J. and Chen, T.-H., DNA evidence for the hybridization of wild turtles in Taiwan: possible genetic pollution from trade animals, *Conserv. Genet.*, 2010, vol. 11, no. 5, pp. 2061–2066.

Frolov, S.V., *Izmenchivost' i evolyutsiya kariotipa lososevykh ryb* (Variability and Evolution of Karyotype of Salmon Fishes), Vladivostok: Dal'nauka, 2000.

Fujisawa, T. and Barraclough, T.G., Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets, *Syst. Biol.,* 2013, vol. 62, pp. 707–724.

Gardner, J.P.H. and Skibinski, D.O.F., Historical and size-dependent genetic variation in hybrid mussel populations, *Heredity*, 1988, vol. 61, pp. 93–105.

Genovart, M., Natural hybridization and conservation, *Biodiversity Conserv.,* 2008, vol. 18, no. 6, pp. 1435–1439.

Gerber, A.S., Tibbets, C.A., and Dowling, T.E., The role of introgressive hybridization in the evolution of the *Gila robusta* complex (Teleostei: Cyprinidae), *Evolution*, 2001, vol. 55, no. 10, pp. 2028–2039.

Glemet, H., Blier, P., and Bernatchez, L., Geographical extent of Arctic char (*Salvelinus alpinus*) mtDNA introgression in brook char populations (*S. fontinalis*) from Eastern Quebec Canada, *Mol. Ecol.*, 1998, vol. 7, no. 12, pp. 1655–1662.

Grant, P.R. and Grant, R.B., Hybridization of bird species, *Science,* 1992, vol. 256, pp. 193–197.

Greenfield, D.W. and Greenfield, T., Introgressive hybridization between *Gila orcutti* and *Hesperolecus symmetricus* (Pisces: Cyprinidae) in the Guyama River basin, California. I. Meristics, morphometrics, and breeding, *Copeia*, 1972, vol. 4, pp. 849–859.

Greenfield, D.W., Abdel-Hameed, F., Deckert, G.D., and Finn, R.R., Hybridization between *Chrosomus erythrogaster* and *Notropis cornutus* (Pisces: Cyprinidae), *Copeia,* 1973, vol. 1, pp. 54–60.

Hanzawa, N., Taniguchi, N., and Shinzawa, H., Genetic Markers of the Artificial Hybrids between *Tribolodon hakonensis* and *T.* sp. (Ukeguchi-Ugui), *Otsuchi Mar. Res. Cent. Rep. Univ. Tokyo,* 1984, vol. 10, pp. 11–17.

Heath, D.A., Rawson, P.D., and Hilbish, T.J., PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada, *Can. J. Fish. Aquat. Sci.,* 1995, vol. 52, pp. 2621–2627.

Hebert, P.D.N., Cywinska, A., and Ball, S.L., Biological identifications through DNA barcodes, *Proc. R. Soc. London, Ser. B,* 2003a, vol. 270, no. 1512, pp. 313–321.

Hebert, P.D.N., Ratnasingham, S., and De Waard, J.R., Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species, *Proc. R. Soc. London, Ser. B,* 2003b, vol. 270, suppl. 1, pp. S96–S99.

Hedges, S.B., Marin, J., Suleski, M., Madeline, P., and Kumar, S., Tree of life reveals clock-like speciation and diversification, *Mol. Biol. Evol.,* 2015, vol. 32, pp. 835–845.

Hewitt, G.M., Quaternary phylogeography: the roots of hybrid zones, *Genetica,* 2011, vol. 139, no. 5, pp. 617–638.

Hubbs, C.L., Hybridization between fish species in nature, *Syst. Zool.,* 1955, vol. 4, pp. 1–20.

Hubbs, C., Kuehne, R.A., and Ball, J.C., The fishes of upper Guadelupe River, Texas, *Texas J. Sci.,* 1953, vol. 5, pp. 216–244.

Hubby, J.L. and Throckmorton, D.H., Protein differences of *Drosophila*. II. Comparative species genetics and evolutionary problems, *Genetics*, 1965, vol. 52, pp. 203–215.

Imoto, J.M., Saitoh, K., Sasaki, T., et al., Phylogeny and biogeography of highly diverged freshwater fish species (Leuciscinae, Cyprinidae, Teleostei) inferred from mitochondrial genome analysis, *Gene,* 2013, vol. 514, no. 2, pp. 112–124.

Inoue, K., Takeuchi, Y., Miki, D., and Odo, S., Mussel adhesive plaque protein gene is a novel member of epidermal growth factor like gene family, *J. Biol. Chem.,* 1995, vol. 270, pp. 6698–6701.

Joly, S., JML: testing hybridization from species tree, *Mol. Ecol. Res.,* 2012, vol. 12, pp. 179–184.

Kafanov, A.I., Subfamily Mytilinae Rafinesque, 1815 (Bivalvia: Mytilidae) in Cainozoe of Northern Pacific, in *Fauna i raspredelenie mollyuskov: severnaya Patsifika i Polyarnyi bassein* (Fauna and Distribution of Mollusks: Northern Pacific and Polar Basin), Vladivostok: Dal'nevost. Nauchn. Tsentr, Akad. Nauk SSSR, 1987, pp. 65–103.

Kartavtsev, Yu.Ph., *Molekulyarnaya evolyutsiya i populyatsionnaya genetika* (Molecular Evolution and Population Genetics), Vladivostok: Dal'nevost. Gos. Univ., 2005.

Kartavtsev, Yu.Ph., *Molekulyarnaya evolyutsiya i populyatsionnaya genetika* (Molecular Evolution and Population Genetics), Vladivostok: Dal'nevost. Gos. Univ., 2009a.

Kartavtsev, Y.Ph., Analysis of sequence diversity at mitochondrial genes on different taxonomic levels: applicability of DNA based distance data in genetics of speciation and phylogenetics, in *Genetic Diversity,* Mahoney, C.L. and Springer, D.A., Eds., New York: Nova Science, 2009b, ch. 1, pp. 1–50.

Kartavtsev, Y.Ph., Sequence divergence at mitochondrial genes in animals. Applicability of DNA data in genetics of speciation, phylogenetics and molecular ecology, *Mar. Genomics,* 2011a, vol. 49, pp. 71–81.

Kartavtsev, Y.Ph., Sequence divergence at *Co-1* and *Cyt-b* mtDNA on different taxonomic levels and genetics of speciation in animals, *Mitochondrial DNA,* 2011b, vol. 2, no. 3, pp. 55–65.

Kartavtsev, Y.Ph., Sequence diversity at *Cyt-b* and *Co-1* mtDNA genes in animal taxa proved Neo-Darwinism, *J. Phyl. Evol. Biol.*, 2013a, vol. 1, no. 4, pp. 1–5. http://dx.org/10.4172/2329-9002.1000120.

Kartavtsev, Y.Ph., Some current concerns of Neo-Darwinism: gene introgression throughout a species border, *J. Phyl. Evol. Biol.,* 2013b, vol. 1, no. 5, pp. 1–4. http://dx.org/10.4172/2329-9002.1000e107.

Kartavtsev, Yu.Ph., Genetic divergence of species and other taxa. Geographical speciation and the genetic paradigm of Neo-Darwinism in action, *Usp. Sovrem. Biol.,* 2013c, no. 5, pp. 419–451.

Kartavtsev, Y.Ph., Molecular evolution and population genetics, in *Molecular Evolution and Population Genetics for Marine Biologists*, Boca Raton, Fl: CRS Press, 2015.

Kartavtsev, Y.P., Barcode index number, taxonomic rank and modes of speciation: examples from fish, *Mitochondrial DNA, Part A,* 2018, vol. 29, no. 4, pp. 535–542.

Kartavtsev, Yu.Ph. and Lee, J.-S., Analysis of nucleotide diversity at the cytochrome *b* and cytochrome oxidase 1 genes at the population, species, and genus levels, *Russ. J. Genet.*, 2006, vol. 42, no. 4, pp. 341–362.

Kartavtsev, Y.P., Sviridov, V.V., Sasaki, T., and Hanzawa, N., Genetic divergence of far eastern dace belonging to the genus *Tribolodon* (Pisces, Cyprinidae) and closely related taxa: some insights in taxonomy and speciation, *Russ. J. Genet.,* 2002, vol. 38, no. 11, pp. 1518–1531.

Kartavtsev, Y.Ph., Chichvarkhin, A.Y., Kijima, A., et al., Allozyme and morphometric analysis of two common mussel species of *Mytilus* genus (Mollusca, Mytilidae) in Korea, Japan and Russia waters, *Kor. J. Genet.*, 2005, vol. 27, no. 4, pp. 289–306.

Kartavtsev, Y.P., Park, T.-J., Vinnikov, K.A., et al., Cytochrome *b* (*Cyt-b*) gene sequences analysis in six flatfish species (Pisces, Pleuronectidae), with phylogenetic and taxonomic insights, *Mar. Biol.*, 2007, vol. 152, no. 4, pp. 757–773.

Kartavtsev, Y.Ph., Katolikova, M.V., Sharina, S.N., et al., A population genetic study of the hybrid zone of *Mytilus trossulus* Gould, 1850 and an introduced species, *M. galloprovincialis* Lamarck, 1819 (Bivalvia: Mytilidae) in Peter the Great Bay in the Sea of Japan, *Russ. J. Mar. Biol.*, 2014, vol. 40, no. 3, pp. 208–216.

Kartavtsev, Y.Ph., Sharina, S.N., Saitoh, K., et al., Phylogenetic relationships of Russian Far Eastern flatfish (Pleuronectiformes, Pleuronectidae) based on two mitochondrial gene sequences, *Co-1* and *Cyt-b*, with inferences in order phylogeny using complete mitogenome data, *Mitochondrial DNA*, 2016, vol. 27, no. 1, pp. 667–678.
https://doi.org/10.3109/19401736.2014.913139

Kartavtsev, Yu.Ph., Batishcheva, N.M., Bogutskaya, N.G., et al., Molecular systematics research, DNA barcoding of Altai Osmans, *Oreoleuciscus* (Pisces, Cyprinidae, Leuciscinae), and nearest relatives, inferred from sequences of cytochrome *b* (*Cyt-b*), cytochrome oxidase c (*Co-1*), and complete mitochondrial genome, *Mitochondrial DNA, Part A*, 2017, vol. 28, no. 4, pp. 502–517.

Kartavtsev, Yu.Ph., Sharina, S.N., Chichvarkhin, A.Yu., Chichvarkhina, O.V., Masalkova, N.A., Lutaenko, K.A., and Oliveira, C., Genetic divergence of mussels (Mollusca, Mytilidae) based on the 28S rRNA, 18S rRNA, and H3 nuclear gene sequences, *Russ. J. Genet.*, 2018a, vol. 54, no. 6, pp. 652–669.

Kartavtsev, Y.Ph., Masalkova, N.A., and Katolikova, M.V., Genetic and morphometric variability in the settlements of two mussel species (*Mytilus* ex. gr. *edulis*), *M. trossulus*, and *M. galloprovincialis*, in North-West Japan Sea, *J. Shellfish Res.*, 2018b, vol. 37, no. 1, pp. 1–17.

Katolikova, M., Khaitov, V., and Väinölä, R., Genetic, ecological and morphological distinctness of the blue mussels *Mytilus trossulus* Gould and *M. edulis* L. in the White Sea, *PLoS One*, 2016, vol. 11, pp. 1–25.

Keck, B.P. and Near, T.J., Geographic and temporal aspects of mitochondrial replacement in *Nothonotus darters* (Teleostei: Percidae: Etheostomatinae), *Evolution,* 2009, vol. 64, pp. 1410–1428.

Kijewska, A., Burzynski, A., and Wenne, R., Molecular identification of European flounder (*Platichthys flesus*) and its hybrids with European plaice (*Pleuronectes platessa*), *ICES J. Mar. Sci.,* 2009, vol. 66, no. 5, pp. 902–906.

Kirpichnikov, V.S., *Geneticheskie osnovy selektsii ryb* (Genetic Principles of Fish Breeding), Leningrad: Nauka, 1979.

Kondrashov, A.S., Yampolsky, L.Y., and Shabalina, S.A., On the sympatric origin of species by means of natural selection, in *Endless Forms: Species and Speciation*, Howarth, D.J. and Berlocher, S.H., Eds., Oxford: Oxford Univ. Press, 1998, pp. 90–98.

Mayden, R.L., Chen, W.-J., Bart, H.L., et al., Reconstructing the phylogenetic relationships of the earth's most diverse clade of freshwater fishes—order Cypriniformes (Actinopterygii: Ostariophysi): a case study using multiple nuclear loci and the mitochondrial genome, *Mol. Phyl. Evol.,* 2009, vol. 51, no. 3, pp. 500–514.

Mayer, W.E., Tichy, H., and Klein, J., Phylogeny of African cichlid fishes as revealed by molecular markers, *Heredity,* 1998, vol. 80, no. 6, pp. 702–714.

Mayr, E., *Animal Species and Evolution*, Cambridge: Belknap, 1968.

Mayr, E., Process of speciation in animals, in *Mechanisms of Speciation*, Barigozzi, C., Ed., New York: Alan R. Liss, 1982, pp. 1–20.

McCusker, M.R., Denti, D., van Guelpen, L., et al., Barcoding Atlantic Canada's commonly encountered marine fishes, *Mol. Ecol. Res.,* 2013, vol. 13, pp. 177–188.

McGuire, J.A., Linkem, C.W., Koo, M.S., et al., Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards, *Evolution,* 2007, vol. 61, pp. 2879–2897.

Miya, M., Takeshima, H., Endo, H., et al., Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences, *Mol. Phyl. Evol.,* 2003, vol. 26, no. 1, pp. 121–138.

Miya, M., Saitoh, K., Wood, R., et al., New primers for amplifying and sequencing the mitochondrial ND4/ND5 gene region of the Cypriniformes (Actinopterygii: Ostariophysi), *Ichthyol. Res.,* 2006, vol. 53, pp. 75–81.

Nanney, D.L., Genes and phenes in Tetrahymena, *Bioscience*, 1982, vol. 32, no. 10, pp. 783–788.

Nedunoori, A., Turanov, S.V., and Kartavtsev, Y.Ph., Fish product mislabeling identified in the Russian Far East using DNA barcoding, *Gene Rep.*, 2017, vol. 8, pp. 144–149.

Nei, M., *Molecular Evolutionary Genetics,* New York: Columbia Univ. Press, 1987.

Nei, M. and Kumar, S., *Molecular Evolution and Phylogenetics,* New York: Oxford Univ. Press, 2000.

Nelson, J.S., Hybridization between two cyprinid fishes, *Hybopes plumbea* and *Rhinichthys cataractae*, in Alberta, *Can. J. Zool.,* 1966, vol. 44, pp. 663–968.

Nelson, J.S., Occurrence of hybrids between longnose sucker (*Catastomus catastomus*) and white sucker (*C. comer soni*) in upper Kananaskis Reservoir, Alberta, *J. Fish. Res. Board Can.,* 1973, vol. 30, pp. 557–560.

Nevado, B., Koblmüller, S., Sturmbauer, C., et al., Complete mitochondrial DNA replacement in a Lake Tanganyika cichlid fish, *Mol. Ecol.*, 2009, vol. 18, pp. 4240–4255.

Nevado, B., Fazalova, V., Backeljau, T., et al., Repeated unidirectional introgression of nuclear and mitochondrial DNA between four congeneric Tanganyikan cichlids, *Mol. Biol. Evol.,* 2011, vol. 28, no. 8, pp. 2253–2267.

Oleinik, A.G., Molecular evolution of chars of genus *Salvelinus*: phylogenetic and phylogeographic aspects, *Extended Abstract of Doctoral (Biol.) Dissertation*, Vladivostok: Inst. Mar. Biol., Far Eastern Branch, Russ. Acad. Sci., 2013.

Oleinik, A.G. and Skurikhina, L.A., Mitochondrial DNA diversity and relationships of endemic charrs of the genus *Salvelinus* from lake Kronotskoye (Kamchatka Peninsula), *Hydrobiologia,* 2010, vol. 650, no. 1, pp. 145–159.

Panov, E.N., *Gibridizatsiya i etologicheskaya izolyatsiya u ptits* (Hybridization and Ethological Isolation of Birds), Moscow: Nauka, 1989.

Pardo, B.G., Machordom, A., Foresti, F., et al., Mitochondrial genomics of ostariophysan fishes: perspectives on phylogeny and biogeography, *J. Mol. Evol.*, 2003, vol. 56, pp. 464–472.

Pardo, B.G., Machordom, A., Foresti, F., et al., Phylogenetic analysis of flatfish (order Pleuronectiformes) based on mitochondrial 16S rDNA sequences, *Sci. Mar.*, 2005, vol. 69, pp. 531–543. https://doi.org/10.3989/scimar.2005.69n4531

Penney, R.W. and Hart, M.J., Distribution, genetic structure, and morphometry of *Mytilus edulis* and *M. trossulus* within a mixed species zone, *J. Shellfish Res.,* 1999, vol. 18, no. 2, pp. 367–374.

Pereira, L.H.G., Hanner, R., Foresti, F., and Oliveira, C., Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genetics,* 2013, vol. 14, no. 20, pp. 1–14.

Phillips, R.B. and Zajicek, K.D., Q band chromosomal banding polymorphisms in lake trout (*Salvelinus namaycush*), *Genetics*, 1982, vol. 101, pp. 227–234.

Phillips, R.B., Zajicek, K.D., and Utter, F.M., Q band chromosome polymorphism in Chinook salmon (*Oncorhynchus tshawytscha*), *Copeia*, 1985, vol. 2, pp. 273–278.

Plotner, J., Uzzell, T., Beerli, P., et al., Widespread unidirectional transfer of mitochondrial DNA: a case in western Palaearctic water frogs, *J. Evol. Biol.,* 2008, vol. 21, pp. 668–681.

Podlesnykh, A.V., Brykov, V.A., and Kukhlevsky, A.D., Unstable linkage of molecular markers with sex determination gene in pacific salmon (*Oncorhynchus* spp.), *J. Hered.,* 2017, vol. 108, no. 3, pp. 328–333.

Powell, J.R., Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila, Proc. Natl. Acad. Sci. U.S.A.*, 1983, vol. 80, pp. 492–495.

Prager, E.M. and Wilson, A.C., Slow evolutionary loss of the potential for interspecific hybridization in birds: a manifestation of slow regulatory evolution, *Proc. Natl. Acad. Sci. U.S.A.*, 1975, vol. 72, pp. 200–204.

Ratnasingham, S. and Hebert, P.D.N., BOLD: the barcode of life data system (http://www.barcodinglife.org), *Mol. Ecol. Notes*, 2007, vol. 7, no. 3, pp. 355–364.

Rawson, P.D. and Hilbish, T.J., Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus edulis* species complex, *Mol. Biol. Evol.,* 1995, vol. 12, no. 5, pp. 893–901.

Rawson, P.D., Secor, C.L., and Hilbish, T.J., The effect of natural hybridization on the regulation of doubly uniparental mtDNA inheritance in blue mussels (*Mytilus* spp.), *Genetics,* 1996, vol. 144, pp. 241–248.

Rawson, P.D., Argawal, V., and Hilbish, T.J., Hybridization between the blue mussels *Mytilus galloprovincialis* and *M. trossulus* along the pacific coast of North America: evidence for limited introgression, *Mar. Biol.*, 1999, vol. 134, no. 1, pp. 201–211.

Redin, A.D. and Kartavtsev, Y.Ph., Molecular phylogeny of Russian Far Eastern flatfish (Pleuronectiformes, Pleuronectidae) based on sequences of mitochondrial genes, *Proc. Int. Symp. "Modern Achievements in Population, Evolutionary, and Ecological Genetics," Vladivostok, Vostok Marine Biological Station, September 3–8, 2017*, Vladivostok, 2017, pp. 34–35.

Roberts, D.G., Gray, C.A., and West, D.J., Marine genetic swamping: hybrids replace an obligately estuarine fish, *Mol. Ecol.,* 2010, vol. 19, pp. 508–520.

Roje, D.M., Incorporating molecular phylogenetics with larval morphology while mitigating the effects of substitution saturation on phylogeny estimation: a new hypothesis of relationships for the flatfish family Pleuronectidae (Percomorpha: Pleuronectiformes), *J. Mol. Phyl. Evol.,* 2010, vol. 56, pp. 586–600.

Saarman, N.P. and Pogson, G.H., Introgression between invasive and native blue mussels (genus *Mytilus*) in the central California hybrid zone, *Mol. Ecol.,* 2015, vol. 24, no. 18, pp. 4723–4738.

Saitoh, K., Sado, T., Mayden, R.L., et al., Mitogenomic evolution and interrelationships of the Cypriniformes (Actinopterygii: Ostariophysi): the first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences, *J. Mol. Evol.,* 2006, vol. 63, pp. 826–841.

Saitoh, K., Chen, W.-J., and Mayden, R.L., Extensive hybridization and tetrapolyploidy in spined loach fish, *Mol. Phyl. Evol.*, 2010, vol. 56, pp. 1001–1010.

Sakai, H., Ito, Y., Shedko, S.V., et al., Phylogenetic and taxonomic relationships of northern Far Eastern phoxinin minnows, *Phoxinus* and *Rhynchocypris* (Pisces, Cyprinidae), as inferred from allozyme and mitochondrial 16S rRNA sequence analyses, *Zool. Sci.*, 2006, vol. 23, pp. 323–331.

Sasaki, T., Kartavtsev, Y.P., Uematsu, T., et al., Phylogenetic independence of Far Eastern Leuciscinae (Pisces: Cyprinidae) inferred from mitochondrial DNA analysis, *Gene Gen. Syst.,* 2007, vol. 82, pp. 329–340.

Schwartz, F.J., *World Literature to Fish Hybrids With an Analyses by Family, Species, and Hybrid*, Seattle, Wash: U.S. Dep. Commerce, Natl. Ocean. Atmos. Admin., 1972, vol. 3.

Schwartz, F.J., World literature on to fish hybrids, with an analysis by family, species and hybrid, in *NOAA Technical Report NMFS-750,* Seattle, Wash: U.S. Dep. Commerce, Natl. Ocean. Atmos. Admin., 1981, suppl. 1.

Seehausen, O., Hybridization and adaptive radiation, *Trends Ecol. Evol.,* 2004, vol. 19, pp. 198–207.

Semina, A.V., Polyakova, N.E., and Brykov, V.A., Genetic analysis identifies a cryptic species of Far Eastern daces of the genus *Tribolodon, Dokl. Biol. Sci.,* 2006, vol. 407, no. 1, pp. 173–175.

Setzer, P.Y.V., An analysis of natural hybrid swarm by means of chromosome morphology, *Trans. Am. Fish. Soc.,* 1970, vol. 99, pp. 139–146.

Sharina, S.N. and Kartavtsev, Yu.Ph., Phylogenetic analysis of flatfish (Teleostei, Pleuronectiformes) based on the investigation of nucleotide sequences of cytochrome oxidase 1 gene (*Co-1*), *Russ. J. Genet.*, 2010, vol. 46, no. 3, pp. 401–407.

Shneer, V.S. and Rodionov, A.V., Plant DNA barcodes, *Usp. Sovrem. Biol.*, 2018, vol. 138, no. 6, pp. 531–537.

Simon, R.C. and Noble, R.E., Hybridization in *Oncorhynchus* (Salmonidae). I. Viability and inheritance in artificial crosses of chum and pink salmon, *Trans. Am. Fish. Soc.*, 1968, vol. 97, pp. 109–118.

Sites, J.W. and Marshall, J.C., Operational criteria for delimiting species, *Ann. Rev. Ecol. Evol. Syst.*, 2004, vol. 35, pp. 199–227.

Skibinski, D.O.F., Beardmore, J.A., and Cross, T.F., Aspects of the population genetics of *Mytilus* (Mytilidae: Mollusca) in the British Isles, *Biol. J. Linn. Soc.,* 1983, vol. 19, pp. 173–183.

Skurikhina, L.A., Kartavtsev, Yu.F., Chichvarkhin, A.Yu., and Pan'kova, M.V., Study of two species of mussels, *Mytilus trossulus* and *Mytilus galloprovincialis* (Bivalvia, Mytilidae), and their hybrids in Peter the Great Bay of the Sea of Japan with the use of PCR markers, *Russ. J. Genet.,* 2001, vol. 37, no. 12, pp. 1448–1451.

Smietanka, B., Burzynski, A., Hummel, H., and Wenne, R., Glacial history of the European marine mussels *Mytilus*, inferred from distribution of mitochondrial DNA lineages, *Heredity,* 2014, vol. 113, pp. 250–258.

Smith, G.R., Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates, *Syst. Biol.,* 1992, vol. 41, no. 1, pp. 41–57.

Sneath, P.H. and Sokal, R.R., *Numerical Taxonomy,* San Francisco: W.H. Freeman, 1973.

Spolsky, C. and Uzzell, T., Natural interspecies transfer of mitochondrial DNA in amphibians, *Proc. Natl. Acad. Sci. U.S.A.*, 1984, vol. 81, pp. 5802–5805.

STATISTICA (data analysis software system), version 6, StatSoft, 2001. http://www.statsoft.com.

Steinke, D., Zemlak, T.S., Hebert, P.D.N., and Adamowicz, S.A., DNA barcoding of Pacific Canada's fishes, *Mar. Biol.,* 2009, vol. 156, pp. 2641–2647.

Stevenson, M.M. and Buchanon, T.M., An analysis of hybridization between the cyprinodont fishes, *Cyprinodon variegates* and *C. elegans, Copeia*, 1973, vol. 4, pp. 682–692.

Stoeckle, M.Y. and Thaler, D.S., Why should mitochondria define species? *Hum. Evol.*, 2018, vol. 33, nos. 1–2, pp. 1–30.

STRUCTURE Software. http://pritch.bsd.uchicago.edu/structure.html.

Suzuki, H., Yasuda, S.P., Sakaizumi, M., et al., Differential geographic patterns of mitochondrial DNA variation in two sympatric species of Japanese wood mice, *Apodemus speciosus* and *A. argenteus, Genes Genet. Syst.*, 2004, vol. 79, pp. 165–176.

Suzuki, H., Filippucci, M.G., Chelomina, G.N., et al., A biogeographic view of *Apodemus* in Asia and Europe inferred from nuclear and mitochondrial gene sequences, *Biochem. Genet.,* 2008, vol. 46, nos. 5–6, pp. 329–346.

Takahata, N. and Slatkin, M., Mitochondrial gene flow, *Proc. Natl. Acad. Sci. U.S.A.,* 1984, vol. 81, pp. 1764–1767.

Tamura, K., Stecher, G., Peterson, D., et al., MEGA6: molecular evolutionary genetics analysis version 6.0, *Mol. Biol. Evol.,* 2013, vol. 30, no. 12, pp. 2725–2729.

Tateno, Y., Nei, M., and Tajima, F., Accuracy of estimated phylogenetic trees from molecular data. I. Distantly related species, *J. Mol. Evol.,* 1982, vol. 18, pp. 387–404.

Templeton, A.R., Mechanisms of speciation—a population genetic approach, *Ann. Rev. Ecol. Syst.,* 1981, vol. 12, pp. 23–48.

Templeton, A.R., Species and speciation: geography, population structure, ecology, and gene trees, in *Endless Forms: Species and Speciation*, Howard, D.J. and Berlocher, S.H., Eds., Oxford: Oxford Univ. Press, 1998, pp. 32–43.

Timofev-Resovskii, M.V., Vorontsov, N.N., and Yablokov, A.V., *Kratkii ocherk teorii evolyutsii* (Brief Description of Evolution Theory), Moscow: Nauka, 1977.

Turanov, S.V., Kartavtsev, Yu.Ph., Lipinsky, V.V., et al., DNA-barcoding of perch-like fishes (Actinopterygii: Perciformes) from far-eastern seas of Russia with taxonomic remarks for some groups, *Mitochondrial DNA,* 2014, vol. 40, no. 6, pp. 447–454. https://doi.org/10.3109/19401736.2014.945525

Turanov, S.V., Kartavtsev, Yu.Ph., Lee, Y.-H., and Jeong, D., Molecular phylogenetic reconstruction and taxonomic investigation of eelpouts (Cottoidei: Zoarcales) based on *Co-1* and *Cyt-b* mitochondrial genes, *Mitochondrial DNA,* 2016, vol. 28, pp. 547–557. https://doi.org/10.3109/24701394.2016.1155117

Turner, G.F., Seehausen, O., Knight, M.E., et al., How many species of cichlid fishes are there in African lakes? *Mol. Ecol.*, 2001, vol. 10, no. 3, pp. 793–806.

Väinölä, R. and Strelkov, P., *Mytilus trossulus* in Northern Europe, *Mar. Biol.,* 2011, vol. 58, pp. 815–833.

Vasil'ev, V.P., *Evolyutsionnaya kariologiya ryb* (Evolutionary Karyology of Fishes), Moscow: Nauka, 1985.

Vinnikiov, K.A., Thomson, R.C., and Munroe, T.A., Revised classification of the righteye flounders (Teleostei: Pleuronectidae) based on multilocus phylogeny with complete taxon sampling, *Mol. Phyl. Evol.,* 2018, vol. 125, pp. 147–162. https://doi.org/10.1016/j.ympev.2018.03.01410.1016/j.ympev.2018.03.014

Wang, J., Kuang, Y.Y., Tong, G.X., and Yin, J.H., Genetics analysis on incompatibility of intergeneric hybridizations between *Hucho taimen* (♂) and *Brachymystax lenok* (♀) by using 30 polymorphic SSR markers, *J. Fish. Sci. China,* 2011, vol. 3, pp. 547–555.

Wiens, J.J., Kuczynski, C.A., and Stephens, P.R., Discordant mitochondrial and nuclear gene phylogenies in emydid turtles: implications for speciation and conservation, *Biol. J. Linn. Soc.,* 2010, vol. 99, pp. 445–461.

Xu, L.X., Kuang, Y.Y., Tong, G.X., et al., An analysis on genetic constitution of *Hucho taimen, Brachymystax lenok*, and hybrids (*Hucho taimen* [female] × *Brachymystax lenok* [male]) by SRAP markers, *Acta Agric. Univ. Jiangxiensis*, 2011, vol. 33, pp. 1187–1194.

Yonekawa, H., Moriwaki, K., Gotoh, O., et al., Evolutionary relationships among five subspecies of *Mus musculus* based on restriction enzyme cleavage patterns of mitochondrial DNA, *Genetics*, 1981, vol. 98, pp. 801–816.

Yonekawa, H., Tsuda, K., Tsuchia, K., et al., Genetic diversity, geographic distribution and evolutionary relationships of *Mus musculus* subspecies based on polymorphism of mitochondrial DNA, in *Problemy evolyutsii* (Problems of Evolution), Kryukov, A.P. and Yakimenko, L.V., Eds., Vladivostok: Dal'nauka, 2000, pp. 90–108.

Yang, L., Sado, T., Hirt, M.V., et al., Phylogeny and polyploidy: resolving the classification of Cyprinine 4 fishes (Teleostei: Cypriniformes), *Mol. Phyl. Evol.,* 2015, vol. 85, pp. 97–116.

Zbawicka, M., Wenne, R., and Burzyński, A., Mitogenomics of recombinant mitochondrial genomes of Baltic Sea *Mytilus* mussels, *Mol. Genet. Genomics,* 2014, vol. 289, pp. 1275–1287.

Zbawicka, M., Trucco, M.I., and Wenne, R., Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: hybridization with invasive European *Mytilus galloprovincialis, Genet., Sel., Evol.,* 2018, vol. 50, p. 5. https://doi.org/10.1186/s12711-018-0376-z

Zhang, J., Kapli, P., Pavlidis, P., and Stamatakis, A., A general species delimitation method with applications to phylogenetic placements, *Bioinformatics,* 2013, vol. 29, no. 22, pp. 2869–2876.

Zhokhova, E.V., Rodionov, A.V., Povydysh, M.N., et al., Use of DNA barcoding and DNA fingerprinting for analysis of the quality of medicinal plant materials and medicinal herbal preparations, *Usp. Sovrem. Biol.*, 2019, no. 1 (in press).

Zhuravlev, Y.N. and Avetisov, V.A., The definition of life in the context of its origin, *Biogeosciences,* 2006, vol. 3, pp. 281–291.

Zuckerkandl, E. and Pauling, L., Molecules as documents of evolutionary history, *J. Theor. Biol.,* 1965, vol. 8, no. 2, pp. 357–366.

Translated by V. Mittova