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SHORT COMMUNICATIONS

On the Genetic Uniformity of the Genus *Trichoplax* (Placozoa)

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Abstract—Fragments of the nuclear and mitochondrial genes for the large-subunit rRNA were compared for *Trichoplax* sp. and *T. adhaerens*. High similarity was observed for their sequences, suggesting that different *Trichoplax* isolates belong to one species.

The genus *Trichoplax* includes a single species—*T. adhaerens* Schulze, 1883—which has been discovered in a marine aquarium of Gratz (Austria) [1]. Grell *et al.* [2–4] reexamined *T. adhaerens* with the use of a culture isolated in the Red Sea. *Trichoplax* sp., which presumably originates from the Japanese Sea, has been found in a marine aquarium of Moscow [5]. Histological examination and fine morphological and ultrastructural studies have been performed with *Trichoplax* sp. adults and swarmers of the Moscow culture [6, 7]. High similarity has been demonstrated for *Trichoplax* sp. and *T. adhaerens*. Individuals of the Moscow culture differed only in having larger brown bodies, higher degeneration of brown body-containing cells, and a lack of degenerating cells in the ventral layer [7]. Morphometric and behavioral features are also similar in *Trichoplax* sp. and *T. adhaerens* [8–10]. Yet the identity of *Trichoplax* sp. and *T. adhaerens* is still questionable, because the characteristics available are scarce and the extent of interspecific differences in the genus *Trichoplax* is unknown. At present, the species diversity of the genus *Trichoplax* is still a topical problem in the context of renewed interest in Placozoa as a living model of an ancestor of multicellular animals [11].

Comparison of macromolecules may contribute substantially to the understanding of the relationships between *Trichoplax* sp. of the Moscow culture and *T. adhaerens*. The *Trichoplax* sp. and *T. adhaerens* nuclear genes for the small-subunit ribosomal RNA, whose sequences are available from GenBank [12, 13], differ in only a few (1.6%) nucleotides clustered in 12 sites. Some of these correspond to compression regions and, consequently, could be differently identified because of technical reasons. The actual difference between the two sequences is probably lower than 1.6%. To interpret these data correctly, account must be taken of the fact that the 18S rRNA genes are highly conserved and thereby hardly suitable for estimating the genetic distance between closely related species. A 1–2% difference is characteristic both of individual copies in the genomic family of the repetitive 18S rRNA gene and of the genes of different species. For instance, the difference between human 18S rRNA sequences reported by different authors is almost 1%, while the 18S rRNA genes of placental mammals (human, mouse, and horse) differ by 1–2%. The same degree of similarity has been observed for the most part of the gene between placental animals and marsupials, although the most variable 18S rRNA regions differ to a greater extent [14].

We partly sequenced the mitochondrial and nuclear genes for the large-subunit rRNAs of *Trichoplax* sp. from the Moscow culture. The fragments of interest were amplified in the polymerase chain reaction with oligonucleotide primers developed for Cnidaria mitochondrial DNA [15] or the nuclear 28S rRNA genes [16]. The resulting sequences were deposited in GenBank under accession nos. AY603696 and AY603697.

Of 717 bp of the mitochondrial gene fragment, 14 (2%) were not established unequivocally because of technical reasons or nucleotide polymorphism. Of the other 703 bp, 14 (2%) proved to differ between our sequence and a published partial sequence of the mitochondrial gene for the large-subunit rRNA of *T. adhaerens* [17]. Mitochondrial genes are commonly less conserved evolutionarily than the above nuclear genes for the small-subunit rRNA. For instance, a human large-subunit rRNA gene fragment corresponding to the *Trichoplax* fragment under study shows 5, 7, or 9% difference in comparisons with its chimpanzee, gorilla, and orangutan counterparts, respectively (on evidence of sequence alignment with the BLAST program [18]). Sequence similarity is considerably lower in more distant species. Assuming that all questionable positions differ between *Trichoplax* sp. and *T. adhaerens*, the maximum difference in the mitochondrial genes for the small-subunit rRNA is no more than 4%. The actual difference is probably even lower.

Similar results were obtained for fragments of the nuclear 28S rRNA genes. Of 299 bp that could be compared with two known *T. adhaerens* sequences [19, 20], only two proved to differ. Moreover, a mistake may be assumed for the GenBank entry no. X57253 in one of these cases, because the corresponding nucleotide is identical in the *Trichoplax* sp. fragment and the *T. adhaerens* sequence U65478. This level corresponds to intraspecific differences characteristic of species with the known taxonomic status. For instance, a homology region of the 28S rRNA differs in no more than two positions between different cDNA clones of spur-toed frog *Xenopus laevis* and in three of four positions between *X. laevis* and *X. borealis* or *X. tropicalis,* respectively.

Rather than suggesting a certain taxonomic rank, a particular level of genetic difference indicates only comparatively close relationships for the forms under study. In our case, the observed level testifies that no genetic difference lies behind the morphological and ultrastructural similarity of *Trichoplax* sp. and *T. adhaerens* and that the Moscow culture of *Trichoplax* sp. is probably identical to *T. adhaerens.*

Grell's culture, which was isolated in the Red Sea, and the Moscow culture, which presumably originates from the Japanese Sea, have been studied in most detail and proved to be similar in several genetic markers. In addition, morphologically undistinguishable *T. adhaerens* lines have been found in the Mediterranean [9, 10], in the Bay of Mexico [21], and near the Hawaii [22]. The worldwide distribution of *T. adhaerens* is probably due to swarmers, a migrating form generated via asexual reproduction. Swarmers may adhere to floating substrates and thereby persist in plankton for a long time or be transferred from one basin to another with ballast water of ships [23]. In addition to descriptions of *Trichoplax* forms close to the type species, histological data have been reported for *Trichoplax* sp. collected on the Atlantic coast of France, near the Roskof biological station [24, 25]. Examination of semithin sections stained with iron hematoxylin and azan by the Heidenhain method revealed a greater than in *T. adhaerens* development and a higher thickness of the inner cell layer, which is termed parenchyma and consists, according to Ivanov's interpretation [24, 25], of cells of several types. Since *Trichoplax* sp. is common in the Roskof region [24], it seems necessary to reexamine the Roskof population. Until the relevant data have been obtained, the genus *Trichoplax* can be considered to be monotypic.

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