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Author(s)	Dick, Matthew H.; Mawatari, Shunsuke F.
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Resolving Taxonomic Problems of North Pacific Bryozoans

Matthew H. Dick[1] and Shunsuke F. Mawatari[2]

[1] *COE for Neo-Science of Natural History, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*
[2] *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

ABSTRACT

The North Pacific rim is zoogeogographically complex because it is geographically complex and has a complex geological history. Two present-day routes exist for trans-Pacific dispersal of shelf-dwelling or intertidal marine organisms: the Bering Sea shelf and the Aleutian archipelago. Dispersal has also been intermittently possible between the Pacific and Atlantic; a seaway connected the North Pacific with the Arctic Ocean in the late Miocene, late Pliocene, and repeatedly during the Quaternary. Ice sheets extending onto the southern Alaskan continental shelf during glacial maxima likely formed a barrier to north-south and east-west dispersal of shelf-dwelling benthic animals. The Bryozoa are one of the most diverse components of intertidal and shelf communities around the North Pacific rim and have a substantial fossil record in the region. Thus they are a good group for addressing evolutionary questions, especially the effects of climatic change on marine benthic faunas. However, their utility in such studies is hampered by poorly resolved alpha-level taxonomy. Lack of resolution stems from the paradigm of cosmopolitanism, difficulty in distinguishing intra- from interspecific variation, and an insufficient understanding of which characters are taxonomically informative. Approaches suggested to resolve these problems include a rejection of cosmopolitanism, detailed study of local bryozoan assemblages with adequate illustration of all species encountered, and utilization of DNA sequence data. To illustrate the power of the third approach, preliminary data are presented from a study that examines the correlation between genetic and morphological variation in an Alaskan population of *Rhynchozoon* sp. Two divergent lineages of the 16S mitochondrial ribosomal RNA gene were detected, and selected morpholgical characters examined to date suggest the lineages may also be morphologically distinct.

Keywords: Bryozoa, North Pacific, Taxonomy, DNA sequence, Zoogeography

INTRODUCTION

The North Pacific is zoogeographically complex, partly because it is geographically complex. The northeastern coast of Asia is presently unconnected by any land to North America, yet there are nonetheless potential avenues for dispersal of shelf-dwelling and intertidal marine animals across the North Pacific [1]. One is across the Bering Sea shelf, shallower than 200 m over a vast area between western Alaska and the shelf-break that extends northwestward from Unimak Island in the eastern Aleutians to Cape Navarin, south of the Chukchi Peninsula. Because the Bering Sea shelf is soft-bottomed, it provides an effective barrier to the dispersal of many sessile shelf animals that require hard substrates.

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However, dominant components of Bering Sea bottom communities include mobile populations of large crabs (*Paralithodes* spp. and *Chionoecetes* spp.), and nearly 70 species of gastropod molluscs, of which large neptunids and buccinids make up the bulk of the biomass [2]. The shells of these animals provide hard substrates for other animals, especially bryozoans and hydroids, that would otherwise be unable to inhabit the Bering Sea bottom. Because of ice scouring along its more northern fringes, the Bering Sea constitutes a barrier to the dispersal of predominantly intertidal animals.

Another potential route for amphi-Pacific dispersal of shelf-dwelling, as well as intertidal, animals is along the Aleutian archipelago, which extends some 1,700 km westward from the Alaska Peninsula. Though there are channels deeper than the 200 m shelf-break at a number of points along the archipelago, the greatest distance between any of the islands is 120 km. The western tip of the Aleutians is separated from the Commander Islands off the coast of Kamchatka by 330 km and a trench > 2 km deep. The Commanders are in turn separated from Kamchatka by another 180 km and a trench 5.5 km deep. The ability of animals to accomplish amphi-Pacific dispersal across either the Bering Sea or the Aleutian route depends upon a variety of factors in addition to distances, including sea temperatures, current patterns, and the longevity of larvae. In general, the directionality of dispersals has been poorly documented, though existing data suggest that during the Neogene, migrations from west to east appear to have been more common [3].

The complex present-day geography of the North Pacific is the result of a complex geological history that has both profoundly influenced, and is reflected in, the marine zoogeography of the region. A seaway opened across Beringia in the late Miocene, connecting the Arctic and Pacific Oceans, with a moderate number of trans-Arctic dispersals of marine organisms between the Pacific and the Atlantic Oceans [4, 5]. The seaway was closed during most of the Pliocene, but reopened in the late Pliocene, this time with numerous dispersals of marine species from the North Pacific to the North Atlantic, and fewer in the opposite direction [5]. During the Quaternary Period, sea levels fluctuated with worldwide climatic fluctuations, and the Bering seaway opened and closed several times. During at least the Wisconsin and Illinoian glaciations, when the seaway was closed, continental ice sheets covered the shelf along the southcentral and southeastern Alaskan coast, possibly each time creating a barrier to gene flow between northern and southern populations of boreal shelf species. The cyclical appearances and disappearances of geographic barriers to marine dispersal, coupled with cyclical shifts in animals' latitudinal ranges in response to climate shifts, contributed to present-day zoogeographical patterns.

Attempts to correlate biotic responses of benthic marine organisms with Neogene climatic changes have largely involved studies of molluscs in the North Pacific, and molluscs and corals [e.g., 6, 7] in the tropics. These phyla are useful for such studies because their Recent faunas are relatively well known and, due to calcareous skeletal elements, they have left extensive fossil records. Bryozoans are another group with similar potential, as exemplifed by studies underway in the Neotropics [8, 9]. Like molluscs, bryozoans are one of the most speciose components of Recent shelf and intertidal communities in the North Pacific [1, 10–11]. Furthermore, Neogene and Quaternary deposits bearing fossil bryozoans occur on both sides of the North Pacific, though they appear to be more numerous, or at least better documented, on the Asian [e.g., 12–14] than on the American side [15]. Integrated paleontological, zoogeographic, phylogenetic, and phylogeographic studies of bryozoans thus have the potential to provide data relevant to the responses of North Pacific marine benthic animals to Neogene and Quaternary climate changes and the roles of the Aleutian and Bering Sea routes in trans-Pacific interchange.

The reliability of zoogeographic or evolutionary studies involving bryozoans will ultimately depend on correct species-level, or alpha, taxonomy. Unfortunately, the alpha taxonomy of North Pacific bryozoans is poorly resolved. In this paper, we discuss three difficulties with the alpha taxonomy and suggest ways these might be addressed. As an example of one type of solution, we present the results of a very limited study of genetic variation in a population of *Rhynchozoon* from Ketchikan, Alaska.

RESOLUTION OF TAXONOMIC PROBLEMS IN NORTH PACIFIC BRYOZOANS

Cosmopolitanism

Until recently, many bryozoan taxonomists worldwide functioned under a paradigm of cosmopolitanism. That is, they worked under the assumption that bryozoan species tend to have broad distributions, and they accomodated a huge amount of morphological variation under the umbrella of 'intraspecific geographic variation' [16]. Bryozoologists

working in the eastern Pacific from the 19th to the mid 20th centuries were no exception. In many cases they identified species or species complexes they encountered with the most similar congener described from the Old World.

As an example, Hincks [38] reported *Fenestrulina malusii* (Savigny and Audouin, 1926), originally described from the Red Sea, from the Queen Charlotte Islands, British Columbia. This name was applied by subsequent workers to virtually all specimens of *Fenestrulina* from the eastern Pacific (summarized in the synonymy for *F. malusii* in [17]); partial exceptions were two morphotypes described as "varieties" of *F. malusii* [18–19]. However, in 1995 Soule et al. [20] treated six species occurring in California, none of which they identified with *F. malusii*. They split *Fenestrulina* into two genera, elevated one previously described variety to species status, and described five new species. Other examples of Old World names applied to eastern Pacific species or species complexes include *Cauloramphus spiniferum* (Johnston, 1832), *Microporella ciliata* (Pallas, 1766), *Lagenipora socialis* (Hincks, 1877), and *Parasmittina trispinosa* (Johnston, 1838), to name a few. Even in cases of species such as *C. spiniferum* that actually do have a circumpolar, Arctic-boreal distribution, other eastern Pacific species were included within the presumed range of variation of the broadly distributed species.

A crack in the armor of the paradigm of cosmopolitanism appeared with a study by Soule and Soule [21] on Hawaiian smittinids. Examining morphological characters in fine detail with SEM, the authors treated 22 species of *Parasmittina*, 15 of which they described as new. Bryozoologists around the world were skeptical, thinking that this result perhaps involved hyperactive splitting. However, Jackson and Cheetham [22] subsequently discriminated 15 morphospecies of *Parasmittina* from Panama, and furthermore showed not only that the characters used to identify these species are heritable, but also that the taxa are genetically distinct. In both these studies, the resolution of species complexes involved not the recognition of cryptic species, but rather a correct partitioning of intraspecific and interspecific morphological variation — that is, breaking through the mindset that species tend to be broadly distributed and comprise a large amount of intraspecific variation. Diagnostic morphological characters were there all along.

A couple of centuries of bryozoan taxonomy under the paradigm of cosmopolitanism has had two consequences that affect our understanding of the zoogeography of North Pacific bryozoans. One is that many of the distributional records in the older literature, especially those involving species once viewed as cosmopolitan and for which no illustrations were included in taxonomic monographs, are essentially worthless. Accurate distributional information will accrue only as accurate taxonomy is achieved. The second is that the species diversity of North Pacific bryozoans has been substantially underdocumented [16]. Recent intensive local monographs on both intertidal [10] and subtidal [20] bryozoans in the northeastern Pacific have detected significant proportions of new species, e.g., 25% [10] to 35% [20].

The solution to the problem of cosmopolitanism is, of course, to proceed with bryozoan taxonomy under a working assumption that many species will have rather limited distributions, and to examine and document species with current technologies, using SEM for morphological characters and making increased use of genetic data.

INTRASPECIFIC VERSUS INTERSPECIFIC VARIATION

The opposite problem to that of cosmopolitanism is that species do, in fact, show intraspecific morphological variation among geographically distinct populations. A taxonomist working with a series of specimens of a particular genus collected over a broad geographical range — from California around the Pacific rim to Japan, for example — may have a difficult time in some cases distinguishing intra- from interspecific variation. One way to address this problem is to make intensive collections at selected sites over the geographical range of interest. Some genera in such collections will contain several congeners which can then be recognized as distinct without the confounding factor of geographic variation. Genera that are speciose at particular localities will also tend to be genera that are speciose in the geographic region of interest, and that are taxonomically problematic. As an example, an intensive local study of intertidal bryozoans along 11 km of coast in the vicinity of Kodiak, Alaska [10] identified five species of *Cauloramphus* and four species of *Microporella*. Discrimination of the species in these genera was facilitated by the co-occurrence of congeners, not only at the same locality, but frequently close to one another on the same rock. Furthermore, access to numerous specimens of each species gave an idea of the range of intraspecific variation, including ecophenotypic variation, thus

avoiding the pitfall of describing isolated specimens lying at the endpoints of the range of variation as distinct species. Intensive local studies have the added advantage of providing information on local species diversity, the composition of species assemablages, and species' habitat preferences.

Publications on local, intensive studies of bryozoan faunas should ideally include adequate illustrations of all species encountered, in order to avoid the problem mentioned above of distributional records of nominal species that later proved to be of uncertain identity. To date, only a small proportion of bryozoans in the North Pacific has been illustrated by SEM. A way to begin to remedy this situation without waiting for a massive, region-wide monographic treatment is to publish adequately illustrated local studies. As such studies accumulate, they will shed light on geographic variation among species' populations.

Taxonomically Informative Characters

Another problem closely related to, and perhaps inseparable from, that of distinguishing inter- from intraspecific variation is determining exactly what characters are taxonomically informative in diagnosing species within a genus. As an example, in calloporid bryozoans, the numbers of erect orificial and angled proximal spines are frequently given in species' descriptions, and used implicity or explicitly as diagnostic characters. However, there are indications that spine number may vary considerably among populations of a species. For example, a species of *Cauloramphus* identified as *C. pseudospinifer* Androsova, 1958 at Kodiak, Alaska [10] is very distinct in having more than two avicularia per zooid, some of them in distal positions. Kodiak specimens have 14–18 spines in total, arranged as 5–6 orificial spines and 9–12 proximal spines, whereas at Ketchikan, Alaska farther south, specimens have 16–23 spines in total, arranged as 5–7 orificial spines and 11–17 proximal spines. In this case, the atypical number and placement of avicularia allow unambiguous identification, after which it becomes evident that spine number is not a good diagnostic character.

In many other cases, the relative reliability of putative diagnostic characters may not be so clear. It is probably the case that with enough comparative material collected over broad enough ranges, and enough time, bryozoan taxonomists would be able to resolve this question for any particular taxon. Indeed, this is the comparative approach that has been undertaken for several centuries, and the only feasible approach if there were no other means of assessing the reliability of diagnostic characters. Now, however, taxonomists have access to a source of data — DNA sequences — essentially independent of the morpholological characters traditionally used in taxonomy, and by which the reliability of those characters in species diagnosis can be tested in a non-circular manner. Curiously, bryozoan taxonomists lag far behind their colleagues studying some other phyla in making use of this powerful source of data. The number of published articles using DNA sequence data to address problems in bryozoan taxonomy at any level can at present be counted on scarcely more than the fingers of two hands [9, 23–34]. By comparison, malacologists, despite a traditional reliance on hard-part morphology for taxonomy similar to that of bryozoologists, have embraced DNA sequence data with open arms [e.g., 35], and relevant articles number in the hundreds.

Use of genetic data need not involve massive, broad-scale phylogenetic or phylogeographic studies to produce useful data relevant to problems in bryozoan alpha-level taxonomy. Basically, genetic data can be used to identify distinct mitochondrial lineages at a local or regional scale, and the lineages can then be examined for concordance, or lack thereof, with morpholgoical characters. The detection of suites of clade-specific characters will indicate that the clades are not freely exchanging the nuclear genes governing complex morpholgical characters. In this case, the clades will represent at least morphospecies, and their status as biological species can subsequently be examined. Conversely, if a genetic study shows a single clade to contain different morphotypes that are scarcely diverged from one another genetically, then the morphotypes probably reflect ecophenotypic or astogenetic variation.

One example of a small-scale genetic approach is provided by Schwaninger's [26] study on the complex of *Membranipora membranacea*-like species that occur in the northeastern Pacific. Three similar species of *Membranipora* have been reported in this region: *M. membranacea* (L.) characterized by a negligible, smooth or finely serrate cryptocyst; *M. villosa* Hincks characterized by a finely serrate cryptocyst and having numerous cuticular spines on the frontal membrane and lateral margin; and *M. serrilamella* Osburn, characterized by a markedly serrated, spinous cryptocyst. Yoshioka [36] argued that these nominal species represent a combination of both ecophenotypic variation (induced partly by the presence of nudibranch predators) and astogenetic variation in a single species. He concluded that *M. villosa* and *M. serrilamella* are synonyms of *M.*

membranacea in the northeastern Pacific. More recently, Schwaninger [26] corroborated Yoshioka's results with a phylogeny of *M. membranacea*- and *M. villosa*-like morphotypes from Friday Harbor, Washington and *M. serrilamella* from Japan. Based on 600 base pairs of the mitochondrial COI gene, her phylogeny showed that single mitochondrial lineages include representatives of all three morphs. Furthermore, the three morphs proved to be genetically indistinguishable at the allozyme level, with no fixed allelic differences among them and low θ values, indicating free gene flow. Schwaninger thus concluded that the three morphs, at least in the vicinity of Friday Harbor, are conspecific. Unfortunately, in a failing common to many genetic analyses, she did not include SEM illustrations of the specific morphologies included in her phylogeny.

Another example of a small-scale genetic approach is provided by a study underway in our laboratory. In a field study of intertidal bryozoans at Ketchikan, Alaska in September 2003, what appeared to be a single species of *Rhynchozoon* was found to be common, and 11 specimens were dried for subsequent examination. The specimens showed considerable variation in a number of morphological characters, both within and among colonies. Variation occurred in degree of frontal costation, the nature of processes around the secondary orifice, presence or absence of a frontal umbo, frequency and types of frontal avicularia, and number of spines on marginal zooids. In contrast, characters that appeared much less variable were overall colony form and color, shape of the primary orifice, and form of the ovicell. We performed a simple experiment to test whether these latter, invariant characters were the taxonomically relevant ones by comparing genetic and morphological variation in the Ketchikan population. Our null hypothesis was that the Ketchikan specimens represent a genetically homogeneous population, and that orifice shape and ovicell form are diagnostic characters for this species despite considerable variation in other characters.

Phylogenetic analysis (Fig. 1) of a 430 base-pair fragment of the 16S mtrRNA gene from 7 colonies showed two moderately diverged *Rhynchozoon* clades, designated A and B.

Clade A showed no genetic variation (Table 1), whereas two haplotypes occurred in Clade B, with

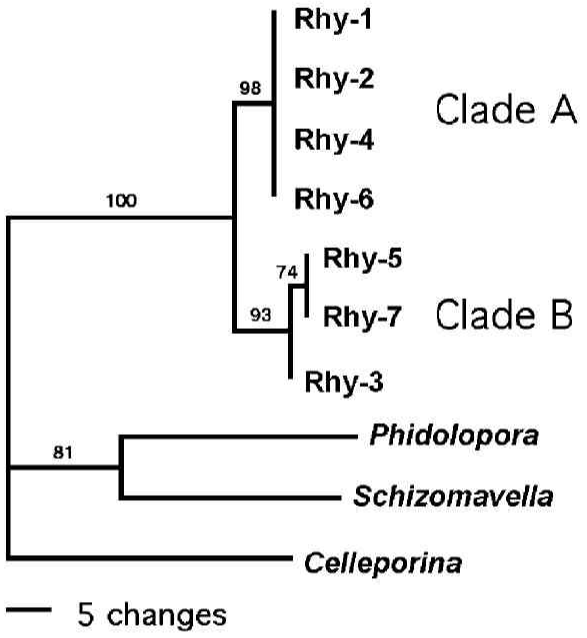

Fig. 1 Single shortest tree from a maximum parsimony analysis of a partial fragment of the 16S mtrRNA gene, including sequences from seven colonies of *Rhynchozoon* sp. (Rhy-1 to 7) and three outgroup taxa; length = 146 steps, CI (excluding uninformative characters) = 0.8765, RI = 0.8864. Values above branches indicate bootstrap values in percent from 1,000 bootstrap replicates, with replacement.

Table 1 Genetic distances (in percent) in pairwise comparisons between separate colonies of *Rhynchozoon* sp. collected at Ketchikan, Alaska. Above diagonal: pairwise "p" distances. Below diagonal, in bold: distances estimated by Kimura's 2-parameter model (K2P) with a discrete approximation of the Γ distribution (K2P + Γ), which are identical to K2P distances after rounding.

	Rhy1	Rhy2	Rhy4	Rhy6	Rhy5	Rhy7	Rhy3
Rhy1	—	0.00	0.00	0.00	2.34	2.34	2.33
Rhy2	**0.00**	—	0.00	0.00	2.34	2.34	2.33
Rhy4	**0.00**	**0.00**	—	0.00	2.33	2.33	2.33
Rhy6	**0.00**	**0.00**	**0.00**	—	2.33	2.33	2.33
Rhy5	**2.38**	**2.38**	**2.38**	**2.38**	—	0.00	0.47
Rhy7	**2.38**	**2.38**	**2.38**	**2.38**	**0.00**	—	0.47
Rhy3	**2.38**	**2.38**	**2.38**	**2.38**	**0.47**	**0.47**	—

an average K2P + Γ distance of 0.31% among the three sequences. Average genetic distance (K2P + Γ) of all pairwise comparisons of sequences between the two clades was 2.38%.

A preliminary examination of morphology indicates that orifice shape (Figs. 2A and 2E), a character traditionally considered important in *Rhynchozoon* taxonomy [17, 37], and ovicell form (Figs. 2B and 2F) appear to be indistinguishable between members of the two clades. In contrast, other characters such as degree of frontal costation (Figs. 2C and 2G) and spine number (Figs. 2D and 2H), though overlapping, are different enough that they can consistently distinguish between the two clades. In other words, there appears to be a suite of morphological characters diagnostic for each clade.

The preliminary data thus do not support our null hypothesis, which was that the Ketchikan specimens of *Rhynchozoon* sp. represent a genetically homogeneous population, and that orifice shape and ovicell form are diagnostic characters for this species despite considerable variation in other characters. Work is underway to examine additional qualitative, as well as quantitative, characters that can distinguish between specimens of the two forms. Elucidation of diagnostic morphological differences between the two will then permit examination of their status as biological species, resolution of their taxonomic status, and investigation of their geographic distributions.

Our results represent the other side of the coin from those of Schwaninger [26]. She did not find *Membranipora* morphology to be congruent with the molecular phylogeny; instead, distinct morphotypes previously accorded nominal species status appear, in fact, to be ecophenotypes of a single species of *Membranipora*. Furthermore, her results indicated deep mitochondrial lineages that nonetheless appear to exchange genes freely at the nuclear level. In contrast, our study indicates a suite of morphological characters congruent with only moderately diverged mitochondrial lineages. The fact that the two lineages appear to have maintained distinct morphologies suggests that they no longer freely exchange the nuclear genes that control suites of complex morphological characters — i.e., that they may well be biological species.

CONCLUSIONS

The North Pacific rim is zoogeographically complex due to its complex geography and geological history; there is a high diversity of Recent bryozoans in intertidal and shelf environments; and sites exist that contain Neogene and Pleistocene bryozoan fossils. Bryozoans in the region are thus a potentially useful group with which to address evolutionary questions, especially those dealing with the effects of climatic change on assemblages of benthic animals. However, the alpha taxonomy of North Pacfic bryozoans is presently inadequately resolved, and this will hamper any such applications. We suggest rejection of the paradigm of cosmopolitanism, adoption of a research strategy to study local assemblages in detail, and utilization of genetic data as ways to improve taxonomic resolution. To demon-

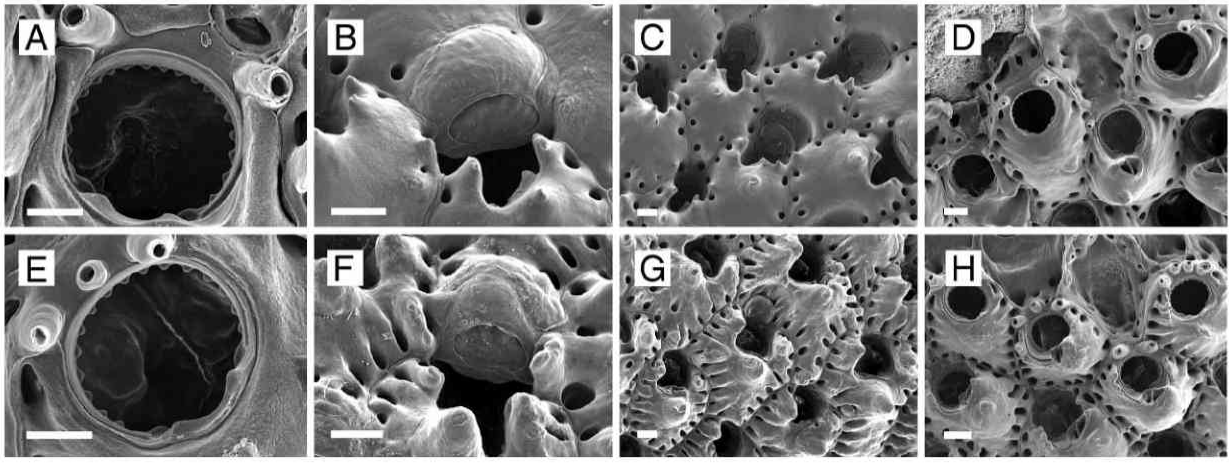

Fig. 2 Comparison of morphology of specimens from *Rhynchozoon* Clades A and B from Ketchikan, Alaska. Top row, Clade A; Bottom row, Clade B. A, E, primary orifice; B, F, ovicell; C, G, degree of costation on frontal wall; D, H, spine number. A, D, specimen Rhy-4; B, C, specimen Rhy-6; E, specimen Rhy-7; F-H, specimen Rhy-3. Scale bar: A,E, 50 μm; B-D and F-H, 100 μm

strate the utility of these approaches, we have presented preliminary results from a small-scale study involving the use of genetic data to differentiate two morphologically distinct lineages of *Rhynchozoon* from a single site in Alaska.

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