

***Cheilonereis shishidoi* (Izuka, 1912) is the correct taxonomic name for the nereidid polychaete species associated with hermit crabs along the Russian coast of the Sea of Japan**

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ABSTRACT. The article presents molecular genetic analysis and data on the ecology and distribution of the symbiotic polychaete genus *Cheilonereis* Benham, 1916 (Polychaeta: Phyllodocida: Nereididae), which is associated with hermit crabs in the southern part of Primorye (mainly in the Peter the Great Bay and Posyeta Bay of the Sea of Japan). The article also provides a valid taxonomic name for this species.

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KEY WORDS: Diversity, symbiosis, associations, Decapoda, Polychaeta, hermit crab, the Sea of Japan, Asia, Northern Pacific

***Cheilonereis shishidoi* (Izuka, 1912) — корректное таксономическое название для полихет-нереид, ассоциированных с раками-отшельниками вдоль российского побережье Японского моря**

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РЕЗЮМЕ. В статье приводятся молекулярно-генетический анализ, а также данные по экологии и распространению симбиотических полихет рода *Cheilonereis* Benham, 1916 (Polychaeta: Phyllodocida; Nereididae), ассоциированных с раками-отшельниками в южной части Приморья (в основном, в заливах Петра Великого и Посъета Японского моря). Статья предлагает также валидное таксономическое название для этого вида симбиотических полихет.

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КЛЮЧЕВЫЕ СЛОВА: Разнообразие, симбиоз, ассоциации, Decapoda, Polychaeta, раки-отшельники, Японское море, Азия, Северная часть Тихого океана.

Introduction

According to the WoRMS database, the genus *Cheilonereis* Benham, 1916 (Polychaeta: Nereididae) currently includes only two commensal species, *C. cyclurus* (Harrington, 1897) (the type species of the genus) from Northern Pacific and *C. peristomialis* Benham, 1916 from Tasmania and South Australia. These species are usually found inside empty gastropod shells occupied by large shallow-water hermit crabs (Martin, Britayev, 1998; Bakken, Wilson, 2005; Read, Fauchald, 2017).

The symbiotic nereidid *Cheilonereis cyclurus* (Harrington, 1897) was described by Harrington (1897) as *Nereis cyclurus* from the Puget Sound in Alaska, USA; it is a common polychaete species found in shallow waters along both the eastern and western coasts of the North Pacific (Harrington, 1897; Izuka, 1912; Berkeley, Berkeley, 1948; Okuda, 1950; Ushakov, 1950, 1955; Imajima, 1972; Paik, 1977; Wu *et al.*, 1985; Marin, 2016, 2020; Marin, Sinelnikov, 2016; Jung *et al.*, 2018). It is usually associated with large hermit crabs from the family Paguridae (Decapoda: Anomura), namely of *Pagurus ochotensis* Brandt, 1851, *P. pectinatus* (Stimpson, 1858) and *P. brachiomastus* (Thallwitz, 1891) in the waters of the Sea of Japan (see Uchida *et al.*, 1963; Marin, Sinelnikov, 2016; Jung *et al.*, 2018), as well as *Pagurus aleuticus* (Benedict, 1892), *P. ochotensis*, *Pagurus armatus* (Dana, 1851), *Paguristes turgidus* (Stimpson, 1857) and *Elassochirus tenuimanus* (Dana, 1851) along the American coast from Gulf of Alaska to California (Harrington, 1897; Clark, 1956; Hickok, Davenport 1957; Seaborn, 1975; Hoberg *et al.*, 1982). This species is also reported as a free-living, scavenging polychaete (Hoberg *et al.*, 1982).

At the same time, a similar species in ecology, *Nereis shishidoi* Izuka, 1912, was described from Yuriage, Miyagi Prefecture of Japan, also in association with large hermit crabs of the genus *Pagurus* Fabricius, 1775 (Paguridae). However, Okuda (1950) synonymized this species with *C. cyclurus* (see) for the following reasons: (1) the structures of its head and feet are very similar to those of *C. cyclurus*, and (2) slight differences in the number of paragnaths are insufficient for specific distinction between *C. cyclurus* and *C. shishidoi* (after Park, 2018).

In addition, a comparison of partial mitochondrial COI sequences revealed significant genetic divergence between *Cheilonereis* specimens from the east and west North Pacific coasts (Park *et al.*, 2017). Based on morphological examination of topotype materials and molecular comparison, Park (2018) reinstated *Cheilonereis shishidoi* (Izuka, 1912) as a valid species, using topotype material from of Asian and American coasts. Nevertheless, these data were only presented in a dissertation (PhD thesis) submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Faculty of the School of Biological Sciences at Seoul National University in South Korea (see Park, 2018). Therefore, these data have actually gone unnoticed in the world scientific literature, and so far all polychaetes associated with hermit crabs along the eastern (Asian) coast of the Northern Pacific are still referred to as *Cheilonereis cyclurus* (e.g., Jimi, 2019; Wilson *et al.*, 2023; Villalobos-Guerrero *et al.*, 2024). Since the publication of the taxonomic change in the dissertation did not comply with the publication criteria set by the International Commission on Zoological Nomenclature (ICZN) (Chapter 8), *C. shishidoi* has continued to be listed as a junior synonym of *C. cyclurus* in the international WoRMS database (Read, Fauchald, 2024).

The aim of this article is to provide a molecular genetic analysis of the symbiotic polychaete worms of the genus *Cheilonereis*, which are associated with hermit crabs along the Russian coast of the Sea of Japan, as well as the indication of a valid taxonomic name for this symbiotic polychaete species.

Material and methods

The study of hermit crabs was conducted in the offshore area of Primorye, from north-western coast of the Sea of Japan from Cape Povorotnyi (42°40'38"N 133°02'33"E) to the southern border of Posyeta Bay, the Tumannaya River (Tumangan) (42°17'37"N 130°42'02"E), during 2012–2020 years. Symbiotic polychaetes were extracted (washed out) from the shells occupied by hermit crabs using magnesium solution. The collected symbiotic polychaetes were then photographed alive in a laboratory using a Canon G16 digital camera. Total body length (tbl., in mm) is defined as the dorsal length from distal prostomium margin to distal margin of most posterior segment.

All collected material is deposited in the Zoological Museum of Moscow University (ZMMU), and

the author's personal zoological collection in the A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences (LEMMI) in Moscow, Russia. This research does not involve any endangered or protected species.

For the molecular-genetic analysis, a fragment of the mitochondrial cytochrome oxidase C subunit I gene (COI mtDNA) was used. Total genomic DNA was extracted from abdominal and pereopod muscle tissue using the innuPREP DNA Micro Kit (AnalytikJena, Germany) following the standard protocol. The gene marker was amplified using the universal primers LCO1490 and HC02198 (Folmer *et al.*, 1994) with the T100 amplifactor (Bio-Rad, USA), under the standard protocol conditions. A consensus of complementary sequences of the COI mtDNA gene marker was obtained using MEGA 7.0 (Kumar *et al.*, 2016), including available COI sequences of the representatives of the genus *Cheilonereis* from Queen Charlotte Sound (51°25'48.0"N 128°42'36.0"W), British Columbia, Canada (HM473330) and from Goseong-gun, Gangwondo, Korea (MF538532) taken from the GenBank (NCBI) database, and from Gajin Harbor, Korea, presented by Park (2018) (CheGa1–3; vouchers NIBRIV0000787932–NIBRIV00007879324). The final dataset included 16 sequences, with 592 base pairs in length, displaying 111 variable (polymorphic) sites, of which 20 were parsimony informative.

Pairwise uncorrected genetic divergence (*p*-distances) was calculated using MEGA 7.0 with the Kimura 2-Parameter (K2P) model of evolution (Kimura, 1980) based on the obtained sequences.

Median joint network (Bandelt *et al.*, 1999) was reconstructed using PopArt (Population Analysis with Reticulate Trees) software (Leigh, Bryant, 2015).

Results

Class Polychaeta Grube, 1850
 Order Phyllodocida Dales, 1962
 Family Nereididae Blainville, 1818
Genus *Cheilonereis* Benham, 1916

Cheilonereis Benham, 1916: 138.

TYPE SPECIES. *Nereis cyclurus* Harrington, 1897.

INCLUDED SPECIES. *Cheilonereis cyclurus* (Harrington, 1897) (type species), *Ch. peristomialis* Benham, 1916, *Ch. shishidoi* (Izuka, 1912), *reinst.*, *comb.n.* (see below).

DIAGNOSIS. Ventral peristomial flap present, covering areas VII–VIII when proboscis everted; conical paragnaths present on both maxillary and oral ring except area V. Dorsal notopodial ligule markedly elongate, broader on posterior chaetigers; notopodial prechaetal lobe present, smaller than dorsal notopodial ligule on anterior chaetigers, restricted to

a limited number of anterior chaetigers; dorsal cirrus mid-dorsally to subterminally attached to notopodial dorsal ligule on posterior chaetigers, lacking basal cirrophore. Neuropodial postchaetal lobe absent or present. Notoaciculae absent from chaetigers I and II. Notochaetae: homogomph spinigers and falcigers. Upper neurochaetae: homogomph spinigers, heterogomph falcigers, blades serrated. Lower neurochaetae: heterogomph spinigers, heterogomph falcigers with long blades on anterior chaetigers, medium blades on posterior chaetigers (after Park, 2018; modified from Bakken, Wilson, 2005).

REMARKS. The genus *Cheilonereis* can be easily distinguished from other nereidid species due to its unique morphological features, such as the ventral peristomial flap at oral ring, it has a unique enlarged collar-like extension of the peristomium that extends around the base of the prostomium. Phylogenetically, based on complete mitogenome sequencing, the genus within the family Nereididae is grouped and closely related to the clade containing *Perinereis nuntia* (Lamarck, 1818), *Perinereis aibuhitensis* (Grube, 1878) and *Platynereis dumerilii* (Audouin et Milne Edwards, 1833) (Bakken, Wilson, 2005; Park *et al.*, 2017).

Cheilonereis shishidoi (Izuka, 1912), *reinst., comb.n.*

Fig. 1B–D.

Nereis shishidoi Izuka, 1912: 177–178, pl. 19, figs 10–18 [type locality — Yuriage, Miyagi Prefecture, Japan].

Cheilonereis cyclurus: Okuda, 1950, 52–53; Uschakov, 1955: 213–214, fig. 67A–E; Uschakov, Wu, 1965: 201; Imajima, 1972: 50–53, figs 6, 7; Park *et al.*, 2017: 669–672, fig. 1AB. For full synonymy see Park (2018).

MATERIAL EXAMINED. Russian Federation, Sea of Japan, Primorskiy Krai (Primorye): 1 specimen (tbl. 56 mm), ZMMU, Peter the Great Bay, Vostok Bay, near scientific station “Vostok”, 42°51'14.48"N 132°46'47.24"E, depth 6 m, deep mud bottom, from the shell occupied by *P. ochotensis*, coll. I. Marin, 12 July 2011; 1 specimen (tbl. 35 mm), LEMMI, same locality and date, from the shell occupied by *P. brachiomastus*; 1 specimen (tbl. 51 mm), LEMMI, Furuhelm Island, northeastern small bay, 42°27'40.7"N 130°55'19.0"E, depth 5 m, from the shell occupied by *P. ochotensis*, SCUBA, coll. I. Marin, A. Maiorova, 15 July 2012; 1 specimen (tbl. 51 mm), LEMMI, Posyeta Bay, Trinity Bay (Bukhta Troitzky), Andreevka, 42°40'4.79"N 131°7'9.24"E, depth: 2–3 m, from the shell occupied by *P. pectinatus*, coll. I.N. Marin, June 2011; 1 specimen (tbl. 43 mm), ZMMU, Sakhalin, Busse lagoon, 46°30'56.2"N 143°18'27.6"E, depth 1–2 m, from the shell occupied by *P. ochotensis*, coll. I. Marin, 23 July 2013; 1 specimen (tbl. 43 mm), ZMMU, Posjeta Bay, Vitjaz, 42°36'7"N 131°10'43"E, depth 1–2 m, from the shell occupied by *P. ochotensis*, coll. I. Marin, 14 June 2015; 1 specimen (tbl. 38

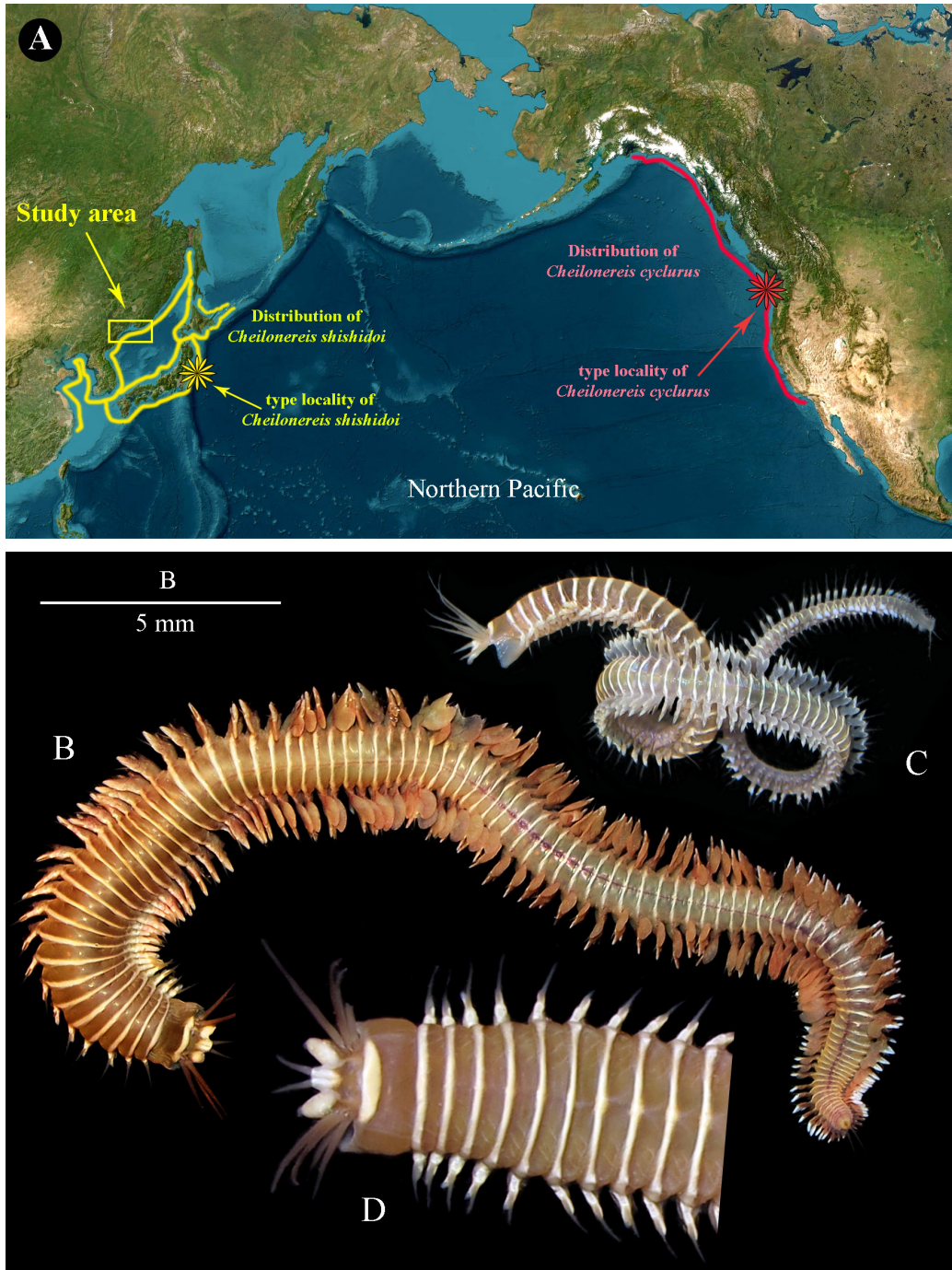


Fig. 1. The known distribution of the genus *Cheilonereis* Benham, 1916 in the North Pacific (A) and alive coloration of *Cheilonereis shishidoi* (Izuka, 1912) comb.n. (B, C — general body; D — head, dorsal view).
 Рис. 1. Известное распространение рода *Cheilonereis* Benham, 1916 в северной части Тихого океана (A) и прижизненная окраска *Cheilonereis shishidoi* (Izuka, 1912) comb.n. (B, C — общий вид; D — голова, вид сверху).

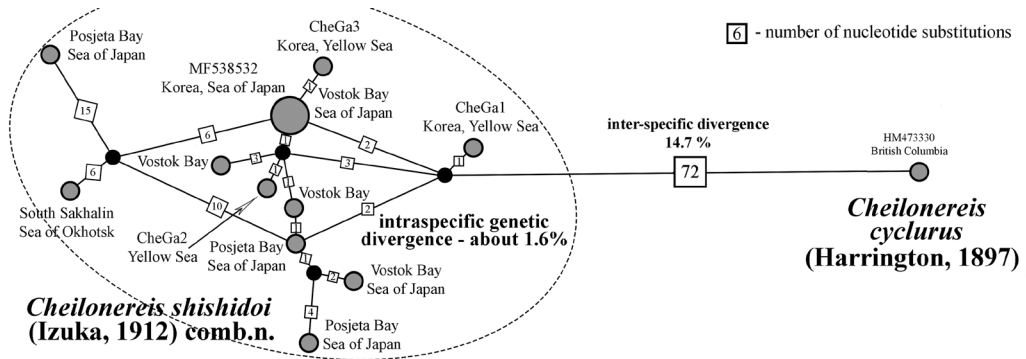


Fig. 2. Median-joint network (TCS) of the studied haplotypes of the species of the genus *Cheilonereis* Benham, 1916 in the North Pacific.

Рис. 2. Сеть (MJ, TCS) изученных гаплотипов видов рода *Cheilonereis* Benham, 1916, обитающих в северной части Тихого океана.

mm), LEMMI, the Peter the Great Bay, Astafieva Bay, 42°36'52.2"N 131°12'01.1"E, depth 1–1.5 m, from the shell occupied by *P. brachiomastus*, coll. Marin, 10 July 2019.

DIAGNOSIS. Similar to the genus (see above).

MORPHOLOGICAL REMARKS. *Cheilonereis shishidoi* can be separated from *C. cyclurus* based on the presence of notopodial homogomph falcigers but only from posterior chaetigers (see Park, 2018). For some other morphological differences see the description presented by Park (2018: figs 22–24).

GENBANK ACCESSION NUMBERS. PQ323322–PQ323329.

MOLECULAR-GENETIC ANALYSIS. The presented comparison of COI sequences between the specimens of *Cheilonereis shishidoi* (Izuka, 1912), reinst., comb.n. from the eastern coast of Korea (see above) and *C. cyclurus* from British Columbia in Canada revealed marked interspecific genetic differences for about 17.3% (p -distance = 0.173) (after Park, 2018). According to our analysis, the specimens collected from the Russian coasts of the Sea of Japan are genetically diverged from the specimen of *C. cyclurus* from British Columbia for about 14.7% (p -distance = 0.147), while the genetic divergence from the Korean specimens is about 1.4% (p -distance = 0.014).

At the same time, the calculated intraspecific genetic divergence within the studied population of *Cheilonereis shishidoi* (Izuka, 1912), reinst., comb.n. ($n=11$) living along the Russian coast of the Sea of Japan is about 1.9% (p -distance = 0.019) and about 0.5% (p -distance = 0.005) in the Korean population ($n=4$). These results suggest that the genetic differences between the Russian and Korean populations can be considered as intraspecific (see Fig. 2).

ECOLOGY. The species is usually found inside the empty gastropod shells that are occupied by large hermit crabs, such as *Pagurus ochotensis*, *P. pectinatus* and *P. brachiomastus* (e.g., Uchida *et al.*,

1963; Marin, Sinelnikov, 2016; Jung *et al.*, 2018). Usually, a single adult worm inhabits a single host mollusk shell occupied by a hermit crab (Marin, Sinelnikov, 2016).

DISTRIBUTION. Type locality: Yuriage, Miyagi Prefecture, Japan. The species has also been recorded in the Sea of Japan and the south-western part of the Sea of Okhotsk Sea, Yellow Sea, both sides (the Sea of Japan and the Pacific Ocean) of the northern Japanese islands (see Fig. 1).

Remarks on other large nereidid polychaetes associated with hermit crabs

Cheilonereis cyclurus (Harrington, 1897) is known as a commensal of large shallow-water hermit crabs *Pagurus aleuticus* (Benedict, 1892), *P. ochotensis*, *P. armatus* (Dana, 1851), *Paguristes turgidus* (Stimpson, 1857) and *Elassochirus tenuimanus* (Dana, 1851) (Decapoda: Paguridae) along the American coast from Gulf of Alaska to California (Harrington, 1897; Clark 1956; Hickok, Davenport 1957; Seaborn, 1975; Hoberg *et al.*, 1982).

Cheilonereis peristomialis Benham, 1916 is reported as a commensal of the mollusk shells occupied by hermit crab *Pagurus edwardsii* (Dana, 1852) (= *Eupagurus edwardsii*) from Tasmania and South Australia (Young, 1923).

Neanthes fucata (Savigny, 1822) lives inside empty gastropod mollusk shells inhabited by hermit crabs *Pagurus prideaux* Leach, 1815 and *Pagurus bernhardus* (Linnaeus, 1758) in the north-eastern Atlantic Ocean, the North Sea and the Mediterranean Sea (Charrier, 1921; Gilpin-Brown, 1969; Martin, Britayev, 1998).

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