Cothurnia polydorica sp. nov. (Ciliophora: Peritrichia), a symbiont of shell-boring spionid polychaetes in the Sea of Japan, and a review of ciliates associated with polydorids

Cothurnia polydorica sp. nov. (Ciliophora: Peritrichia), симбионт полихет – спионид, сверлящих раковины в Японском море, и обзор инфузорий, живущих на полидорах

A.W. Jankowski

А.В. Янковский

A.W. Jankowski, Zoological Institute, Russian Academy of Sciences,1 Universitetskaya Emb., St Petersburg 199034, Russia. E-mail: ciliofora@mail.ru

Small unidentified spionid polychaetes (*Polydora* sp.) boring in shells of gastropod and bivalve molluscs in the Nakhodka region are hosts of a new species of loricate peritrich named *Cothurnia polydorica* **sp. nov.** The large genus *Cothurnia* can be subdivided into two groups (subgenera or genera) with one or two zooids inside the lorica; *C. polydorica* is a monozoic (solitary) species; after cell division one of the daughter individuals becomes migratory and swims away. The trophont is not vermiform and thus is not typical of most cothurnids; contraction of fixed specimens is partial, in the adoral half only; the lorica has a second inner layer of material at its base; the stalk is very long as compared with other cothurnids. The migrants adhere to the tips of thick setae of the host but not to its body surface, on which there are scattered small parasitic rhynchodids sucking the host body fluid by short apical tubes. A review of ciliates associated with polydorids is given.

Мелкие неопределенные полихеты-спиониды (*Polydora* sp.), сверлящие раковины брюхоногих и двустворчатых моллюсков районе Находки, – это хозяева нового вида раковинных перитрих *Cothurnia polydorica* **sp. nov.** Крупный род *Cothurnia* можно разделить на 2 группы (подроды или роды) с 1 или 2 зооидами внутри раковины; *C. polydorica* – монозойный вид; после деления клетки одна особь становится мигрантом и уплывает. Трофонт не червеобразный, нетипичный для большинства котурнид; сокращение при фиксации частичное, в антесоме; раковина двойная в основании; стебелек удлинен. Мигранты прикрепляются к вершинам щетинок, а не к телу хозяев, где рассеяны мелкие паразитические ринходиды, высасывающие тканевую жидкость с помощью короткой апикальной трубки. Дан обзор цилиат, живущих на полидорах.

Key words: Polychaeta, Spionidae, *Polydora*, Ciliophora, Peritrichia, *Cothurnia*, new species

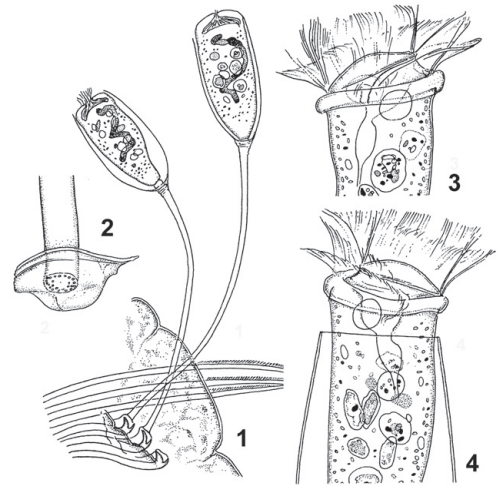
Ключевые слова: Polychaeta, Spionidae, *Polydora*, Ciliophora, Peritrichia, *Cothurnia*, новый вид

INTRODUCTION

In 1987 and 1988 periphyton and symbiotic ciliates were studied in the environs of the Biological Station “Vostok” of the Far Eastern Institute of Marine Biology near Nakhodka City, on the Asian coast of the Sea of Japan. Several new species and genera were discovered, including the new species described below which occurred only on “blister-worms,” or shell-boring polychaetes.

MATERIAL AND METHODS

Molluscs were collected in summer 1988 on sublittoral mussel and oyster beds; shells of living bivalves and gastropods were crushed to extract boring worms; some of them were examined alive, and others were fixed in 4–8% formalin diluted with sea water. Drawings of the new species were made without a camera lucida from living and fixed ciliates. The macronucleus was stained by acidified methyl green purified by a simple new method: about 40–50 drops are dried on a white plastic disc placed in a Petri dish to protect it from dust; the methyl violet contaminant is immediately absorbed into the white plastic due to electrophoresis, and when the dried double stain is redissolved in water, only methyl green is dissolved; the traditional use of chloroform for separation of two stains in solution is thus avoided. The dried drops are dissolved before use when needed, so that one Petri dish can be used for 40–50 subsequent stainings.



Figs 1–4. *Cothurnia polydorica* **sp. nov. 1**, two zooids on typical long stalks attached to setal tips; **2**, adhesive disc of stalk; **3**, **4**, peristomes of two living filtering zooids.

Order **VORTICELLIDA**

Family **VAGINICOLIDAE**

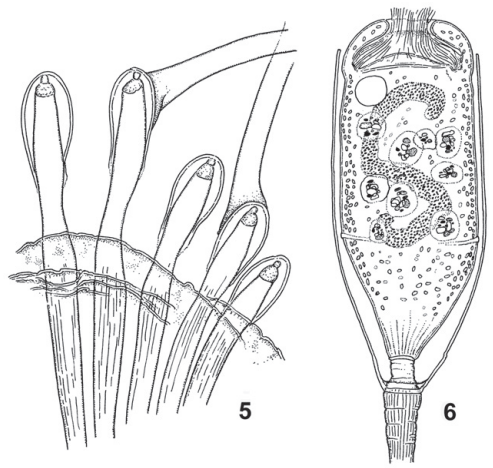
Genus ***Cothurnia*** Ehrenberg, 1831

***Cothurnia polydorica* sp. nov.**
(Figs 1–8)

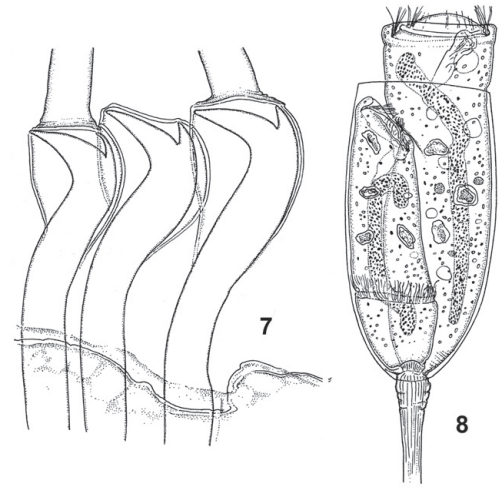
Type material. The type slide is a hapantotype (No. 1988–27); it includes the whole worm with numerous ciliates, in glycerin-formol, framed with black bituminous varnish; this slide and five 10-ml jars containing dozens of worms are stored in the Laboratory of Protozoology of the Zoological Institute of the Russian Academy of Sciences (St Petersburg). One worm, also with numerous ciliates, is stored separately as a *xenotype*; this is my term for the type specimen of the host which was proposed before the widely accepted term *symbiotype* of Donald Duszynski. I insist on my priority and will use my terminology below.

Description. Shell elongate, cup-like, thin walled, translucent, symmetrical, with upper margin not folded outwards. Stalk very long (2–2.5 times as long as body), relatively thin, dense and refractile, with distinct longitudinal striation in its upper part that may be wider than stalk distal to it and may bear irregular transverse folds. Length of lorica (in micrometers, ten specimens): 52–58, width of lorica: 25–28, stalk length: 84–98, stalk thickness: about 3.5. Three distinct zones between scopular (lower) body end and tip of stalk may be distinguished with oil immersion: 1) short endostyle, 2) wide low mesostyle, and 3) base of internal shell between these two zones (Figs 6, 8). Stalk attaching to substrate by wide folded basal disc.

Well-fed zooid before fission fills entire shell (Fig. 6); body wide, 60–72 μm long, gray due to numerous mitochondria; its contours not resembling those of typical vermiform cothurnids and zooids of *Platycola* Kent, 1882 and *Thuricola* Kent, 1881, species with cylindrical body shape. Peristome of usual type, with thick distinct circular lip, with 1.5 turns of peristomal ciliated spiral and with relatively short vestibulum; contractile vesicle single, below lip. Cilia long; at Fig. 8 only basal parts of cilia are shown. Macronucleus looking like longitudinal curved band, typical of other cothurnids; micronucleus not discernible among numerous refractile mitochondria



Figs 5, 6. *Cothurnia polydorica* **sp. nov. 5**, attachment sites of two zooids (only stalk bases are shown) on tips of setae; **6**, fixed zooid (methyl green stain), showing the nucleus, mitochondria and adhesive disc; note restricted contractility of the fixed cell.



Figs 7, 8. *Cothurnia polydorica* **sp. nov. 7**, bases of stalks on tips of the host's uncinate setae; **8**, differentiation of two daughter cells after cell division into trophont and migrant; methyl-green nuclear staining.

and reserve grains. Pellicular cross-striations that are distinct in most cothurnids were not discerned in this species but they are likely to be present; no silver staining was made. Unlike mobilids, all the species of sessilids without exceptions possess argentophilic striations; the surface reticulum (like that of *Pseudovorticella* Foissner & Schiffmann, 1975) is unknown in loricates.

This species has a limited contractility. Figs 6 and 8 were made from specimens stored in seawater-formalin; large zooids do not contract entirely inside shells when fixed, and remain almost unchanged except for contraction in their peristomal regions. Thin myonemes are seen above the scopula (Fig. 6).

Comparison. Two groups of species can be distinguished in *Cothurnia* s. l., with one or two active zooids on the joint endostyle. I have introduced special terms for the shelled (loricate) peritrichs, distinguishing *monozoic* and *dizoic* species and genera. The new species clearly belongs to the monozoic forms that are united in the genus *Cothurnia* Ehrenberg, 1831, s. str., as opposed to the numerous dizoic cothurnids of the genus *Sincothurnia* Jankowski, 1985. After fission (Fig. 8), one of the two daughter cells continues feeding while the other contracts its peristome, develops a submedial ciliated ring and after metamorphosis swims away to settle on free uninhabited tips of setae of the same or another host. Thus, there can be no objections to the inclusion of the new species in *Cothurnia*.

The periphyton at the sampling site includes only one monozoic cothurnid (*Cothurnia maritima* Ehrenberg, 1838) with a relatively short and thin stalk, and with a different shape of the zooid and lorica. There is no reason to suppose that it can settle on the cryptic polydores and change its shape. Symbionts of polychaetes are host specific species with definite morphological characteristics; this article adds a new type of loricate symbiont to those previously known on *Harmothoe* Kinberg, 1856, *Polynoe* Savigny, 1818, *Nereis* Linnaeus, 1858 and *Arenicola* Lamarck, 1801.

Cothurnid ciliates were monographed by Kahl (1935) and Warren & Paynter (1991); the symbiont of the polydores differs from all the previously known species

by its cylindrical body shape and by its extremely long stalk; in both freshwater and marine cothurnids the exostyle is short, only rarely reaching the body length, whereas in the new species it can be twice as long as the body and longer. The cothurnid keys of Warren & Paynter (1991) should be modified to include this long-stalked species.

Etymology. The species name is based on the name of its host, the genus *Polydora* Bosc, 1802.

Host. The host of the new species is an unidentified spionid of the genus *Polydora* boring in shells of large bivalves *Crenomytilus grayanus* (Dunker, 1853), *Crassostrea gigas* (Thunberg, 1793) and large patellid limpets, an unidentified species of the genus *Acmaea* Eschscholtz, 1833. Almost all the examined worms (near 150 specimens) from the shells of these molluscs bore at least two or three, maximum 45 peritrichs. They were localized on the tips of massive setae of two kinds (Figs 1, 5, 7), never on the body surface, on which small ancistrocomid ectoparasites from the order Rhynchodida were randomly scattered, feeding by sucking body fluids through small rigid apical tubes.

A review of ciliates associated with the polychaete family Spionidae

Spionid polychaetes (now near 1000 species) are tiny free-living (usually in dense clusters of mud tubes) and boring worms; the latter ones can be extracted from coralline algal crusts, large barnacles, coral blocks, shells of living molluscs, empty shells and (like *Polydora commensalis* Andrews, 1891) pagurid-inhabited shells (Blake & Evans, 1973); some species are symbionts of sponges (*P. colonia* Moore, 1907, *P. spongicola* Berkeley & Berkeley, 1950), tunicates and other invertebrates. The main polydorid genera are *Polydora* Bosc, 1802 , *Dipolydora* Verrill, 1879, *Tripolydora* Woodwick, 1964, *Pseudopolydora* Czerniawski, 1881, *Boccardia* Carazzi, 1893, *Boccardiella* Blake & Kudenov, 1978, *Carazziella* Blake & Kudenov, 1978 (Blake & Kudenov, 1978).

The Russian fauna was treated by Uschakov (1955; outdated monograph), and mainly by Radashevsky (1993, 1994, and a series of previous and subsequent articles), who discovered many new species; for my research area (Peter the Great Bay) he reported, of the strictly polydorid subgroup of Spionidae, the genera *Boccardiella* [*B. hamata* (Webster, 1899)], *Pseudopolydora* [*P. kempi japonica* Imajima & Hartman, 1964, *P. orientalis* (Annenkova, 1937) = *Carazzia orientalis*] and 14 species of the genus *Polydora* including more than 70 species worldwide; four of these were described by Radashevsky. The review of polydorids in Northwest Pacific (Radashevsky, 1993) includes about 30 species. His exciting discovery of *P. vulcanica* Radashevsky, 1994, that makes extensive mats in sulfur-rich volcanic caldera in the Mid-Kuriles reminds us that this group of worms had not attracted due attention of protozoologists; only a few ciliate symbionts were described from this family with worldwide distribution while much more hosts await careful study.

A few examined spionids are hosts of intestinal ciliates (Astomatida), ectoparasitic ancistrocomids (Rhynchodida), urceolarians (Mobilida) and sedentary peritrichs (Sessilida) of two types, with or without lorica. In fact, the associates of spionids are similar to those of sabellids, cirratulids and terebellids, except for the absence of vermigemmid Suctoria which were common on the two latter families in my NW Pacific samples.

A large astome named *Anoplophrya polydorae* De Faria, Da Cunha & Da Fonseca, 1917 was found in the intestine of *Polydora socialis* (Schmarda, 1861) extracted from *Ostrea* Linnaeus, 1758 shells in Brazil (De Faria et al., 1917, 1918); since the type of *Anoplophrya* Stein, 1860 is a dissimilar parasite of nordic earthworms, the generic name of the spionid parasite was later changed (Puytorac, 1954). Puytorac (1955a, 1955b) described a new microsporidian of the ge-

nus *Thelohania* Henneguy, 1892, that infects both the host tissues and the intestinal astome ciliate *Hovassiella polydorae* Puytorac, 1955 in *Polydora giardi* Mesnil, 1896; similar astomes in cirratulids, both freeliving and shell-borers (like *Dodecaceria concharum* Oersted, 1843), were treated in detail in his monograph of the order Astomatida and in a later article (Puytorac, 1954; Puytorac & Schrevel, 1965: *Durchoniella cirratuli* Puytorac, 1954 and *D. dodecaceriae* Puytorac & Schrevel, 1965).

Precht (1935) described a solitary peritrich *Scyphidia spionicola* Precht, 1935from *Pygospio elegans* Claparede, 1863 and from some other unidentified spionid in northern Germany. *Scyphidia* Dujardin, 1841 is a false genus with no determinable type species: *S. rugosa* Dujardin, 1841 from freshwater benthos was poorly described in an older work (Dujardin, 1841) and was never redescribed; thus the generic position of this symbiont should also be changed. A similar stalkless species with a discoid attachment, but with a compact nucleus, was found by me on polydores on the Murmansk shore of the Barents Sea.

Douglas & Jones (1991) examined nine species of spionids and some other polychaetes in Southern California, including *Polydora* (3), *Boccardia* (2) and one species of each of the genera *Pseudopolydora*, *Pygospio* Claparede, 1863, *Streblospio* Webster, 1879 and *Scolelepis* Blainville, 1828 (subgenus *Nerinides* Mesnil, 1896). In all, they found 26 species of parasites, including ancistrocomids on all the worms except *Boccardia proboscidea* Hartman, 1940, one astome species, and (only on *Polydora ligni* Webster, 1879) one mobilid, possibly urceolarian; three non-polydorids were free of these symbionts. Their illustrations give no possibility of identification of these symbionts. A species of *Urceolaria* Stein, 1867 occurred on nearly 90% of *P. ligni*, and its presence was enough for host identification. Astomes infected nearly 50% of *P. nuchalis* Woodwick, 1953, *P. socialis* (Schmarda, 1861) and *Scolelepis maculata* (Hartman, 1961); they may be "*Anoplophrya*" *polydorae* or some other species. Ancistrocomids, superficially similar, infected *Boccardia hamata* (near 100%), *Polydora nuchalis* (100%), *P. ligni* (40%), *P. socialis* (40%), and only 1% of *Pseudopolydora paucibranchiata* (Okuda, 1937). Three spionids (*Boccardia proboscidea* Hartman, 1940, *Pygospio elegans* Claparede, 1863 and *Streblospio benedicti* Webster, 1879) were free of ciliates.

Commensals may be seen on illustrations (light and SEM photographs) of worms, for example on the setae of *Dipolydora armata* (Langerhans, 1880) boring in the calcareous hydrozoan *Millepora complanata* Lamarck, 1816 on coral reef in Barbados, West Indies (Lewis, 1998). Fig. 4 A of the cited author shows not "spermatophores, 50 μm long, attached to setae of female worms", as stated in the legend, but typical small peritrichs on short stalks, with note in the text: 40–50 μm long; with stalk 20–30 μm long; attached by stalk to capillary setae of genital segments of females, occasionly also to body surface (body wall) of females. This is in fact a new undescribed peritrich, perhaps *Rhabdostyla* sp.

When describing the cosmopolitan spionid *Dipolydora armata* (Langerhans, 1880) from the epifauna of the gastropod *Thais haemastoma* (Linnaeus, 1767) from the Ibiza coast of the Mediterranean (extracted from empty shells or those inhabited by pagurids *Calcinus* Dana, 1851and *Clibanarius* Dana, 1852), Bick (2001) includes a photograph of a symbiont, with the legend "Fig. 7 B. Peritrichous ciliate (*Cothurnia* sp.) on capillary /seta/ of postbranchiate notopodium", and with two in-text notes. Page 181: "Peritrichous ciliates (*Cothurnia* sp.) were sometimes attached to the notopodial capillaries on setigers immediately following branchiate setigers, both in April and September. Usually one ciliate was attached to a bundle, rarely two specimens". Page 186: "The occurence of peritrichous ciliates on the notopodial spines of postbranchiate setigers was remarkable and echoes findings of Williams & Radashevsky (1999) in *Polydora*

neocaeca Williams & Radashevsky, 1999. Setae are clearly a suitable substratum for sessile ciliates. The constant water current produced by ciliated branchiae could improve the conditions for feeding and growth of symphoriontic Ciliophora. The occurence of sessile unicellular organisms on setae may be a common phenomenon and is worthy of further investigation".

Unless reflected in the abstracts, such records of symbionts may be easily overlooked in the extensive literature on polydores and other polychaetes; I will therefore be grateful for such references. The occurence of an unidentified mobilid *Urceolaria* sp. was noted in the abstract of a thesis (David, 2011) and in a published article (David & Williams, 2012). This article adds new species to the relatively small order Urceolariida with a restricted number of hosts; SEM shows a small ciliate with several medial rings or folds, which are not typical of urceolarians that have either a smooth or a completely ringed lateral body surface. The host *P. colonia* Moore, 1907 makes numerous tubes inside sponges in the West Atlantic and in the Mediterranean Sea; the examined worms were extracted from the sponges *Microciona prolifera* (Ellis & Solander, 1786) and *Halichondria bowerbanki* Burton, 1930 in New York region, where *P. colonia* may be an introduced, non-native species.

Small immobile ancistrocomids on polydorids and sabellids may belong to the genus *Colligocineta* Kozloff, 1965 (Kozloff, 1965, 1976; Raabe, 1970), with the type species *C. furax* Kozloff, 1965 on sabellids *Laonome kroyeri* Malmgren, 1866. Besides two species on *Sabella pavonina* Savigny, 1822 (*C. affinis* Kozloff, 1976 and *C. finleyi* Kozloff, 1976), the genus includes also *C. scolelepidis* Kozloff, 1976 from the spionid *Scolelepis fuliginosa* (Claparede, 1870). A typical *Colligocineta* was common in my samples of *Polydora ciliata* Johnston, 1838, living in mud tubes on sandy littoral (estuarine mudflats) on the Murmansk coast of the Barents Sea.

ACKNOWLEDGEMENTS

I am grateful to the reviewer Prof. John C. Clamp (University of North Carolina, USA) for valuable comments and numerous corrections to the submitted manuscript.

REFERENCES

Bick A. 2001. The morphology and ecology of *Dipolydora armata* (Polychaeta, Spionidae) from the western Mediterranean sea. *Acta Zoologica* (Stockholm), **82**(3): 177–87.

Blake J.A. & Evans J.W. 1973. *Polydora* and related genera as borers in mollusk shells and other calcareous substrates (Polychaeta: Spionidae). *The Veliger*, **15**(3): 235–249.

Blake J.A. & Kudenov J.D. 1978. The Spionidae (Polychaeta) from south-eastern Australia and adjacent areas with a revision of the genera. *Memoirs of the National Museum of Victoria*, **39**: 171–280.

David A.A. 2011. Natural history, reproduction and regeneration of the sponge associate *Polydora colonia* (Polychaeta: Spionidae) based on specimens from Long Island, New York. Master of Science Thesis, Hofstra University, Hempstead, New York State.

David A.A. & Williams J.D. 2012. Morphology and natural history of the cryptogenic sponge associate *Polydora colonia* Moore, 1907 (Polychaeta: Spionidae). *Journal of Natural History*, **46**(23/24): 1509–1528.

De Faria G., Da Cunha A. & Da Fonseca O. 1917. Sobre es protozoarios parazitos da *Polydora socialis*. *Brasil Medico*, **31**: 243.

De Faria G., Da Cunha A. & Da Fonseca O. 1918. Protozoarios parazitos de *Polydora socialis*. *Memorias do Instituto Oswaldo Cruz*, Rio de Janeiro, **10**: 3–4 (in English), 17–19 (In Portuguese).

Douglass Th.G. & Jones I. 1991. Parasites of California spionid polychaetes. *Bulletin of Marine Science*, **48**(2): 308–317.

Dujardin F. 1841. Histoire naturelle des Zoophytes. Infusoires, comprenant la physiologie et la classification de ces animaux, et la maniere de les etudier a l'aide du microscope. Paris: Libairie Encyclopedique de Roret. 684 p.

Kozloff E. N. 1965. *Colligocineta furax* gen. nov., sp. nov., an ancistrocomid ciliate (Holotricha: Thigmotricha) from the sabellid polychaete *Laonome kroyeri* Malmgren. *Journal of Protozoology*, **12**: 333–334.

Kozloff E.N. 1976. New ancistrocomid ciliates from polychaete annelids. *Transactions of the American Microscopical Society*, **95**: 622–627.

Lewis J.B. 1998. Reproduction, larval development and functional relationships of the burrowing spionid polychaete *Dipolydora armata* with the calcareous hydrozoan *Millepora complanata*. *Marine Biology*, **130**(4): 651–662.

Precht H. 1935. Epizoen der Kieler Bucht. *Nova Acta Leopoldina Carol. Deutsche Akademie der Naturforschung, Halle. Neue Folge*, **3**: 405–474.

Puytorac P. de. 1954. Contribution a l'étude cytologique et taxonomique des Infusoires Astomes. *Annales des Sciences Naturelles, Zoologie*, Série 11, **16**: 85–270.

Puytorac P. de. 1955a. Sur *Hovassiella polydorae* nov. gen., n. sp., cilié astome endoparasite de *Polydora giardi* Mesn. *Archives de Zoologie Expérimentale et Générale*, **93**: 20–34.

Puytorac P. de. 1955b. Présence simultanée d'une microsporidie *Thelohania georgevitchi* sp. nov. dans les cellules intestinales de *Polydora giardi* M. et dans un cilié parasite du tube digestif de ce même polychéte. *Comptes Rendus Hebdomaires de l'Académie des Sciences de Paris*, **240**: 925–927.

Puytorac P. de & Schrevel J. 1965. Nouvelles espéces de ciliés astomes endoparasites d'annélides polychétes. *Annales de la Faculté des Sciences, Université de Clermont-Ferrand*, **26**: 85–99.

Raabe Z. 1970. Ordo Thigmotricha (Ciliata – Holotricha). III. Familiae Ancistrocomidae et Sphenophryidae. *Acta Protozoologica*, **7**: 385–463.

Radashevsky V.I. 1993. Revision of the genus *Polydora* and related genera from the North West Pacific (Polychaeta: Spionidae). *Publications of the Seto Marine Biological Laboratory*, **36**(1/2): 1–60.

Radashevsky V.I. 1994. Species of the genus *Polydora* (Polychaeta, Spionidae) from the Middle Kuril Islands. *Bulletin of the National Science Museum, Tokyo, Series A, Zoology*, **20**(2): 67–76.

Uschakov P.V. 1955. Polychaete worms of the far eastern seas of the USSR (Polychaeta). Keys to the fauna of the USSR, edited by Zoological Institute, Academy of Sciences of the USSR, **56**: 1–445. (In Russian).

Warren A. & Paynter J. 1991. A review of *Cothurnia* (Ciliophora: Peritrichida) and its morphological relatives. *Bulletin of the British Museum (Natural History), Zoology Series*, **57**: 17–59.

Williams J.D. & Radashevsky V.I. 1999. Morphology, ecology and reproduction of a new *Polydora* species (Polychaeta: Spionidae) from the East Coast of North America. *Ophelia*, **51**(2): 115–127.

Received June 20, 2014 / Accepted September 14, 2014

Editorial responsibility: D.A. Gapon