New species of the genus *Sagitta* sensu str. (Chaetognatha) from the Sea of Japan with description of an original staining and dissection technique

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A detailed description, figures of a new species *Sagitta sublica* sp. nov. and the original techniques of research are presented. A key of species and subspecies of the world fauna of the *Sagitta* sensu str. Quoy et Gaimard, 1827 (for individuals at maturation stages III-V) is done.

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Introduction

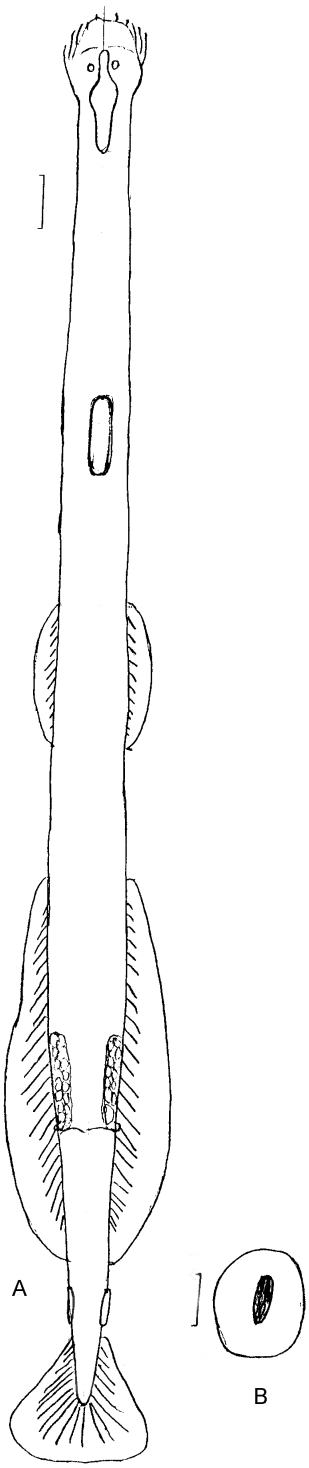
The aim of the paper is to present the original methods of staining and dissection technique and to describe a new species of *Sagitta* from the Sea of Japan. Diagnoses and lists of species for all genera of Sagittidae was done by Kassatkina (2007). A key to the genera of Sagittidae has been published by Kassatkina (2006, 2007). The diagnoses and key of species for all species of *Sagitta* s. str. are presented now.

Techniques of research

Staining technique. To see a ciliated loop of chaetognaths is rather difficult. In fresh-caught species it can be observed while constantly changing the intensity of light. In the fixed individuals it is easier to make it with the help of a staining technique developed in 1971 (Kassatkina, 1971, 1982). In a ciliated loop between two its rings (external and internal) there is a rather deep groove which is kept even if the loop cells laying on a body surface are damaged. It is necessary to place stain just into this groove.

It is possible to stain with both methylene-blue and carmine. The surface subjected to staining, should be necessarily free of water and fixing agent, otherwise the stain will be dissolved in them, that is equivalent to immersing the whole individual into stain that does not give desirable result as it is stained simultaneously both a ciliated loop, and skin. Before staining it is necessary to prepare tools: a dissecting needle with the bent end for keeping an animal, and a thin brush, which end is cut so that 2-3 hairs are projecting. A brush is sopped into stain and its projecting hairs touch a back surface of an animal between eyes and on a neck. The needle for this purpose is poorly suitable, as from it a big drop of stain is usually falling, while at staining by a brush it does not take place. On a surface first of all there are well seen protruding parts: many sensorial-locomotory bodies surrounding a ciliated loop, and a ciliated loop itself. Within several minutes a loop, and sensorial-locomotory bodies are well visible on a background of poorly stained skin; then visibility of the loop sharply worsens, as the skin, at last, is stained over.

Frequently, it is necessary to stain not only a loop, but also lateral fins. It is caused by the fact that at the majority of species the sheet is compressed as a result of fixing, fins are immersed into lateral fields, and their forward and back ends become invisible on the ventral and dorsal sides. The measurement of the animal fins in such position gives wrong sizes. Meanwhile the sizes of fins, their remoteness from belly ganglia, and also from each other, represent important systematic features. To reveal difficultly distinct forward and back ends of pair fins we do the following. An animal is turned on a lateral surface and is hold with the bent end of a needle. Stain is rendered precisely the same as on a ciliated loop – a touch of the brush end to that area of lateral fields where the fin settles down. Staining is carried out under



Figs 1-2. *Sagitta sublica* sp. n. **1**, habitus; **2**, eye. Scale bar: 1 mm (A), 0.05 mm (B).

binocular, the most convenient magnification is about 8 × 2.

Technique of dissection. A body of the majority of Chaetognatha species is dense, but transparent – the gut and ovaries are well visible. However, in the region of a neck at many species of *Parasagitta*, *Sagitta* and *Omittosagitta* muscles are so strongly developed and are so condensed at fixing, that it is impossible to see a median gut. Observation in transmitted light often misleads relative to both form of the gut coat diverticulums and their presence (intestinal diverticulums is a genetic feature, therefore, it is especially important to reveal them). False notion about the presence of intestinal diverticulums is sometimes created. It is a result of the fact that the longitudinal condensed muscular tissue, attached to the caudal end of skeletal head plates, forms two semicircles on both sides of a gut. As they are in the area of intestinal diverticle curves, then in the transmitted light a zone of muscular bands attachment can be easily accepted for the forward end of intestinal diverticulums. The enlightenment of covers and muscles by glycerin does not give necessary effect as the muscular bands are clarified worse, than the paries of a median gut and intestinal diverticulums. In such cases, only prosection can reveal intestinal diverticulums on a median gut and head gut. Prosection is carried out by dissecting needles made of entomological pins (no. 0, 1 and 2) which are bent and sharpened. An animal is put the belly side upwards and is hold with the left needle. Sharpened end of the other needle is entered inside the covers of a body and it is done a medial cut in the direction of a head. The cut should not be short as the diverticulums entrances of the median gut at some species settle down at a distance of 1-1.5 head length. Therefore, the cut should be started at the distance of approximately 1-1.5 head length from a neck. After the skin-muscular sac is opened down to a neck, a dissecting needle should be entered under a zone of muscular bands attachment to lateral plates of a head and they should be cut so that it is possible to turn off the edges and see a median gut. In some *Sagitta* species having a complex structure of the head gut (with the same diverticulums as in a median gut), it is necessary to open the gut itself. For this purpose, first it is cut the skin-muscular sac on a head along the median line, between muscles (m. Complexus lateralis) which in part are turned off for observation of a head gut. Then, similarly it is opened the gut itself by entering a needle inside the gut and cutting it along the median line.

Recommendations on measurement. Animals are measured without a loose plate of a caudal fin. It is measured only that part of length of a tail fin which lays on a caudal part. Measurement of the caudal part, also the parts of fins of the 2-nd pair

laying on the truncal and caudal parts, is done from the bottom margin of the ovaries spermatheca. This is related to the peculiarities of ways of Chaetognatha insemination - the size from spermatheca up to a tail plate is important at spermatophore transport from a tail fin to spermatheca.

Sagitta sublica sp. n.
(Figs 1-2)

Holotype. Inventory no. SA-3-P-15, Sea of Japan, 42°30′N, 132°00′E, R/V "Professor Kaganovskiy", horizon 100-0 m, plankton sample at 20 p.m. using Jeddy net, 11.06.2006; deposited in the collection of Pacific Institute of Oceanography, Far East Division, Russian Academy of Sciences, Vladivostok.

Paratypes. 5 specimens from the same sample as holotype in the same collection.

Description (holotype). Body rigid and muscular, not flagging on a pincette. Head as broad as trunk. Neck hardly noticeable, there is no drastic narrowing in the region of seminal receptacle. Body length 26.5 mm. Tail region 19.6%, ventral ganglion 5.7% of body length. Diverticula are absent. Corona ciliata (long, one pare gyris) situated on trunk two times more than head section, starting from brain. Anterior end of anterior lateral fins located behind a ventral ganglion posterior level.

This distance is 1.7 times as long as the ganglion and makes 6% of the bodylength. Length of anterior fins is 10.5% of body length, 2.6 times shorter than the length of posterior fins. The anterior end of posterior fins is located from the posterior end of anterior fins at a distance equal to that between the ventral ganglion and the anterior fins. Length of posterior fins makes 27.5% of body length; trunk part of posterior fins is 1.92 times larger than tail part. Rays pierce both paired and unpaired fins, but borders of all fins are devoid of rays. Alveolar tissue and sensory-locomotory corpuscules are absent. There is one pair of hook rows and two pairs of dental rows on head: 6 hooks, 5 front teeth, and 15 posterior teeth on each side. Hooks, front and posterior teeth are of a usual shape for *Sagitta.* Shape of Hooks, front and posterior teeth are usual for *Sagitta.* Eyes have an entirely, without excavated pigment spot in center. The specimen is at 3rd maturity stage. Seminal sacks not filled with spermatophore, flattened and elongate, without contact of both pairs of lateral fins and a tail fin. Tail fin is about 5% of body length and 25% of tail region. Ovaries are 6% of body length, containing a few ripe eggs and numerous immature eggs.

Remarks. Discovery of a new species in the north-western part of the Sea of Japan may be related to the water transport from the eastern part of the sea by synoptical vortices which have been being revealed by hydrologists from 1977 till the present time (Lobanov et al.)

Comparison. The new species differs from *S. kussakini* in the position of seminal vesicles relative to both pairs of lateral fins and a tail fin.

A key of species and subspecies of the *Sagitta* sensu str. Quoy et Gaimard, 1827 (for individuals at maturation stages III-V)

1(2). Anterior lateral fins begin above the level of the anterior end of the ventral ganglion . . . . . 1. **S. nagae** Alvarino, 1967
2(1). Anterior lateral fins begin below the level of the anterior end of the ventral ganglion.
3(8). Anterior lateral fins begin level with the middle ventral ganglion.
4(7). Anterior lateral fins are longer than those of the second pair.
5(6). Anterior ends of posterior fins and posterior ends of anterior fins look fused in ventral and dorsal view. However, it is seen in lateral view that they are parallel to each other: anterior ends of posterior fins situated more dorsal than anterior fins in front of their posterior ends. . . . . 2. **S. sceptrum** Kassatkina, 2008
6(5). Anterior ends of posterior fins located far from posterior ends of anterior fins . . . . . 3. **S. pulchra** Doncaster, 1903
7(4). Anterior lateral fins are shorter than those of the second pair in mature animals, and in juveniles their length may be equal . . . . . 4. **S. bedoti** Beraneck, 1895
8(3). Anterior lateral fins begin after the level of the middle of the ventral ganglion.
9(14). Anterior lateral fins begin at the level of the posterior end of ventral ganglion.
10(11). Seminal vesicles touch both lateral and caudal fins . . . . . 5. **S. bruuni** Alvarino, 1967
11(10). Seminal vesicles positioned from either the lateral fins or the caudal fin.
12(13). Seminal vesicles positioned from the lateral fins and touch the caudal fin . . . . . 6. **S. izuensis** Kitou,1966
13(12). Seminal vesicles touch the lateral fins and positioned from the caudal fin . . . . . 7. **S. abyssicola** Chidgey, 1989
14(9). Anterior lateral fins begin below the level of the posterior end of the ventral ganglion.
15(16). The distance between the posterior end of the ventral ganglion and anterior end of anterior lateral fins is less than 1/2 the ganglion length . . . . . 8. **S. bipunctata** Quoy et Gaimard,1827
16(15). The distance between the posterior end of the ventral ganglion and anterior end of anterior lateral fins are 1/2 ganglion length and more.
17(20). The distance between the posterior end of anterior lateral fins and the ventral ganglion is slightly less than the length of ganglion, but more than 1/2 ganglion.
18(19). Seminal vesicles approximate lateral fins and are negligibly remote from the caudal fin. The caudal fin has full rays; there are no rayless zones . . . . . 9. **S. euneritica** Alvarino,1961
19(18). Seminal vesicles are remote from both lateral and caudal fins by a considerable distance, which extends many times the length of the sacs per se. The caudal fin has an inner rayless zone . . . . . .10. **S. modesta** Kassatkina, 1971
20(17). The distance between the posterior end of the ventral ganglion and the anterior lateral fins is longer than the ganglion.
21(22). The distance between the posterior end of the ventral ganglion and the anterior lateral fins exceeds the ganglion length no more than 1.5 times . . . . . .11. **S. nutana** Kassatkina, 1982

22(21). The distance between the posterior end of the ventral ganglion and the anterior lateral fins exceeds the ganglion by length more than 1.5 times.
23(29). The distance between the posterior end of the ventral ganglion and the fins of the first pair is always less (at all maturity stages of animals) than that between the fins of the first and second pairs.
24(26). Seminal vesicles remote from side fins by a distance that exceeds the length of the testicles per.se. In fins, rays are full, without rayless zones . . . . . .12. **S. glacialis** Molchanov, 1907
25a(25b). The relative length of the caudal department exceeding 17%, the first pair of fins exceeding 13%, and the second pair of fins exceeding 19% of the body length . . . . . .12a. **S. glacialis glacialis** Molchanov, 1907
25b(25a). The relative length of the caudal department not exceeding 17%, the first pair of fins not exceeding 13%, and the second pair of fins not exceeding 19% of the body length . . . . . 12b. **S. glacialis baltica** Ritter-Zahony, 1911
26(24). Seminal vesicles positioned from the lateral fins at a much shorter distance than the length of the vesicles themselves, or they contact fins. Fin rays are incomplete, and rayless zones are present.
27(28). Seminal vesicles touch lateral rays and are far from the caudal fin . . . . . 13. **S. setosa** Muller, 1847
28(27). Seminal vesicles are equidistant from lateral fins and the caudal fin . . .14. **S. euxina** Molchanov, 1907
29(23). The distance between the posterior end of the ventral ganglion and the anterior lateral fins is equal to (in immature animals) or exceeds (in mature animals) the distance between fins of pairs I and II.
30(31). Seminal vesicles are far from paired lateral fins and almost touch the caudal fin; an insignificant distance between vesicles and the fin can be seen only at large magnification. A alveolar tissue partly covers vesicles and the caudal fin and fringes the neck. The ciliary loop lacks convexity in the neck region . . . . . .15. **S. kussakini** Kassatkina, 1997
31(30). Seminal vesicles without contact both pairs of lateral fins and a tail fin . . . . . 16. **S. sublica** sp. n.

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