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Taxonomic differences in the microstructure of the integument in males of the genus *Nannocalanus* Sars, 1925 (Calanoida: Calanidae)

Таксономические отличия самцов рода *Nannocalanus* Sars, 1925 (Calanoida: Calanidae) в строении микроструктур интегумента

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КЛЮЧЕВЫЕ СЛОВА: *Nannocalanus*, Calanidae, таксономические особенности, поры интегумента.

ABSTRACT. Males of three species of the genus *Nannocalanus* Sars, 1925 could be identified by the location of integumental pores on the abdomen. Males of two species from the northern Indian Ocean, *Nannocalanus major* Sewell, 1929 and *N. sewelli* Kazus, 2009, differ in the location of pores on the first and second segments of the abdomen. At the same time, both of them clearly differ from *N. minor* (Claus, 1863) from the Mediterranean Sea, which is the type species of this genus. The distinguishing feature for the former two species is one pair of pores on the dorsal side in the middle part of the second, third and fourth segments of the abdomen, which is missing in *N. minor*.

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РЕЗЮМЕ. Особенности расположения пор на интегументе абдомена позволили дифференцировать самцов трех видов рода *Nannocalanus* Sars, 1925. Самцы двух видов из северной части Индийского океана — *Nannocalanus major* Sewell, 1929 и *N. sewelli* Kazus, 2009 — различаются между собой по специфическому расположению пор на первом и втором члениках абдомена. При этом оба индоокеанских вида четко отличаются от *N. minor* (Claus, 1863) из Средиземного моря, являющегося типовым видом этого рода, наличием с дорсальной стороны в срединной части на втором, третьем и четвертом члениках по одной паре пор, которая у *N. minor* отсутствует.

Introduction

Copepods of the genus *Nannocalanus* Sars, 1925 are widely distributed in the World Ocean, mainly in the tropical and subtropical areas but also in the antarctic and sub-antarctic regions [Razouls *et al*., 2005–2025]. For a long time all *Nannocalanus* populations were classified as *N. minor* Sewell, 1929. The second species *N. elegans* Andronov, 2001 has been described in 2001 in the southeastern part of the Pacific Ocean. Two forms from the Indian Ocean first described by Sewell [1929], *N. minor* f. *major* и *N. minor* f. *minor* Sewell, 1929, were later accepted as separate species *N. major* and *N. sewelli* Kazus, 2009 based on the morphology of females [Kazus, 2009]. However, males of these species could not be identified because of their morphological similarities and practically identical structure of their mouth parts and swimming legs [Kazus, 2009]. It wasn't possible to find differences neither in the amount of denticles on the inner edge of the coxopodites of the left and right fifth swimming legs, nor in the ratio of the length of the serrated inner edge of the coxopodite of the fifth swimming leg to its whole length. Sewell [1947] mentioned that he wasn't able to find clear differences between males of these forms except for minor differences in the structure of the fifth pair of swimming legs and antennules.

In this study integumental organ staining method [Alekseev, Naumova, 2005] has been applied to differentiate between *Nannocalanus* males from the northern part of the Indian Ocean and compare them with *N. minor* males from the Mediterranean Sea. Taxonomic significance of the integumental organs has been first shown for closely related Calanoida species by Fleminger [1973] and later significantly expanded and summarized by Mauchline [1987]. Later studies have confirmed that

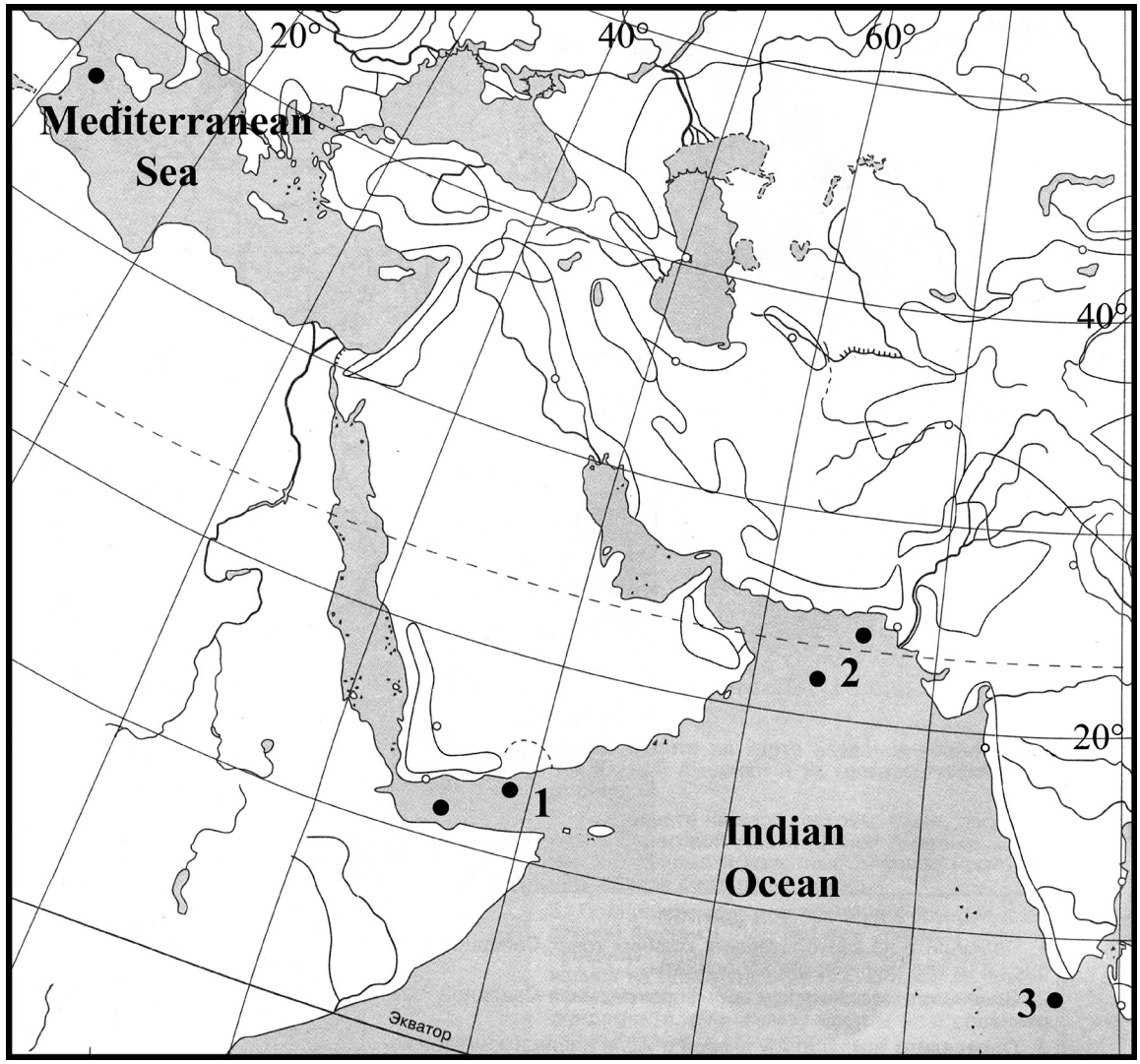

Fig. 1. Sampling regions in the Indian Ocean (1 — Gulf of Aden, 2 — Arabian Sea, 3 — Laccadive Sea) and Mediterranean Sea.

Рис. 1. Район исследования в Индийском океане (1 — Аденский залив, 2 — Аравийское море, 3 — Лаккадивское море) и Средиземном море.

although the informative value of the pore pattern may vary from taxa to taxa they often represent stable, species-specific features, which has been shown for some genera [Koomen, 1992; Prusova, 2003]. The location of the pores is considered to be the most informative on the abdomen, slightly less on the cephalon [Park, Mauchline, 1994]. Therefore the aim of our study was to identify additional morphological features and the species-specific location of integumental pores in morphologically similar male species of the genus *Nannocalanus*.

Material and methods

Specimens of *Nannocalanus* were taken from the Gulf of Aden, the Arabian and Laccadive Seas (depths from 40 to 3000 m) (Fig. 1, Table 1). Zooplankton samples were taken by vertical net hauls from different depths on scientific research voyages of the Azov-Black Sea Institute of Fisheries and Oceanography, and then transferred to us for detailed taxonomic studies. *N. minor* individuals from the Strait of Sicily in the Mediterranean Sea, a place close to its original description, were transferred to us from the collection of the Institute of Biology of the Southern Seas (IBSS, Sevastopol). Samples were stored in a 3–4% buffered formalin solution in seawater.

Body length of all specimens was measured from the anterior edge of cephalon to the posterior border of caudal rami. After that the abdomens of the crustaceans were stained with aniline red dye using the express method of examining the organs of the integument [Alekseev, Naumova, 2005]. First the soft tissues of the crustaceans were dissolved in a drop of sodium hypochlorite solution. After that the exoskeleton was placed in distilled water, and then on a glass slide in a drop of dye. By rapidly heating the slide with a gas lighter the dye was brought to a boil, after which the exoskeleton was transferred to distilled water. To sketch the pores, the abdomen was placed on a slide in a drop of glycerin with a cover glass.

Length of the serrated part of the inner edge of the left coxopodite of the fifth pair of swimming legs to the entire length of this edge ratio was measured in males. The following common abbreviations are used in the article: P5 — 5th pair

Table 1. Sampling information.
Таблица 1. Характеристика исследованного материала.

Water area	Station	Research vessel	Coordinates		Depth, m	Sampling depth, m	Time, h	Date	*Nannocalanus major*, no. of specimens, ♂	*N. sewelli*, no. of specimens, ♂	*N. minor*, no. of specimens, ♂
			Lat.	Lon.							
Arabian Sea	679	"Lesnoy"	24º22′1″	65º50′	356	100–0	14.00	04.01.67	3	3	
	111	"Vorobiev"	21º50′5″	64º35′	3000		8.45		8	3	
Laccadive Sea	789	"Lesnoy"	7º34′6″	77º33′4″	80	80–0	17.00	23.01.67	18	6	
Gulf of Aden	Пр 12	Unknown	11º42′	46º13′	1866		23.00		10		
	108		14º47′5″	49º55′	40	37–0		01.08.71	12		
Strait of Sicily	Пр 72	"Alexandr A. Kovalevskiy"	36º10′5″	12º35′5″	1200	120–52	16.09	14.01.70			2
	Пр 73		36º40′5″	12º35′5″	1200	51–26	16.15	14.01.70			2
	74		36º40′5″	12º35′5″	1200	25–10	16.21	14.01.70			4
	89		36º59′2″	11º35′8″	418	52–26	07.50	16.01.70			2
	99		36º59′2″	11º35′8″	418	11–0	13.15	16.01.70			1
	116		36º59′5″	11º19′8″	220	50–25	00.27	16.01.70			2

of swimming legs, B1 — coxopodite. A total of 93 crustaceans were colored and studied: *Nannocalanus major* ♂ — 51, *N. sewelli* ♂ — 12, *N. minor* ♂ — 30 specimens.

Optical microscopes MBR-1 and MBD-1 were used in this study. Drawings were made with the RA-4 drawing machine.

Results

Analysis of the location and number of external pores on the abdomen of male *Nannocalanus* from the northern Indian Ocean made it possible to divide individuals into two species, since there is a constant number of pores on the chitin of the abdomen with fixed location. It was shown that there was some variability in pore location on the cephalothorax, although the main pattern remained. Thus, the number and location of pores on the abdomen can be identified as a stable morphological feature for distinguishing between closely related species. Some morphological features for each of the three species have been studied (Table 2).

N. major, male (Figs 2A−B, 3A, 4A−B). Total body length 1.46−1.88 mm (average 1.60 mm, n=30). The gnathobase of maxillular praecoxopodite bears 2−4 denticles and one small seta at the base of the first pair of denticles (n = 50). The number of denticles on the inner surface of B1P5 is 12−16 (average 14.0, n=24) for the left coxopodite, and 9−16 (average 12.6) for the right one. The ratio of the serrated part to the entire part of B1P5 is 77−98% (average 81%, n=24).

In the distal part of the first segment of the abdomen (left view), both pores are located at different levels relative to the distal edge of the segment. Dorsally, only one of these pores is visible, approximately in the middle of this segment, always above the chitinous fold. On the second, third and fourth segments of the abdomen (left view), the pores in its median part are located almost at the same level relative to the distal edge of the segment. Dorsally, there is one pair of pores in the middle part of the second, third and fourth segments.

N. sewelli, male (Figs 2C−D, 4C−D). Total body length 1.52−1.60 mm (average 1.52 mm, n=8). The gnathobase of maxillular praecoxopodite bears one small seta at the base of the first pair of denticles, 6 out of 20 examined specimens had 3–4 weakly pronounced denticles.

The number of denticles on the inner surface of B1P5 is 12−14 (average 13.2, n=5) for the left coxopodite, and 10−14 (average 12.2) for the right one. The ratio of the serrated part to the entire part of B1P5 is 78−92% (average 84%, n=6).

In the distal part of the first segment of the abdomen (left view), both pores are located at the same level relative to the distal edge of the segment. Dorsally, only one of these pores is visible, located at the base of the chitinous fold, about 1/3 of the length of this segment from the distal edge. On the second segment of the abdomen (left view), the pores in its middle part are located at different levels relative to the distal edge of the segment. Dorsally, there is one pair of pores in the middle part of the second, third and fourth segments.

N. minor, male (Figs 3B, 4E−F). Total body length 1.62−1.80 mm (average 1.70 mm, n=30). The gnathobase of maxillular praecoxopodite bears 1−2 rows of denticles

Table 2. Morphological characteristics of the three *Nannocalanus* species.
Таблица 2. Морфологические характеристики трех видов *Nannocalanus*.

			N. major	*N. sewelli*	*N. minor*
Body length	Range, mm		1.46−1,88	1.52−1.60	1.62−1.80
	Average, mm		1.60	1.52	1.70
Number of denticles on B1P5	left	Range, mm	12−16	12−14	10−18
		Average, mm	14	13.20	13
	right	Range, mm	9−16	10−14	9−15
		Average, mm	12.60	12.20	12.40
Ratio of the serrated part of the left B1P5 to its whole length	Range, %		77−98	78−92	83−94
	Average, %		80.90	84	90

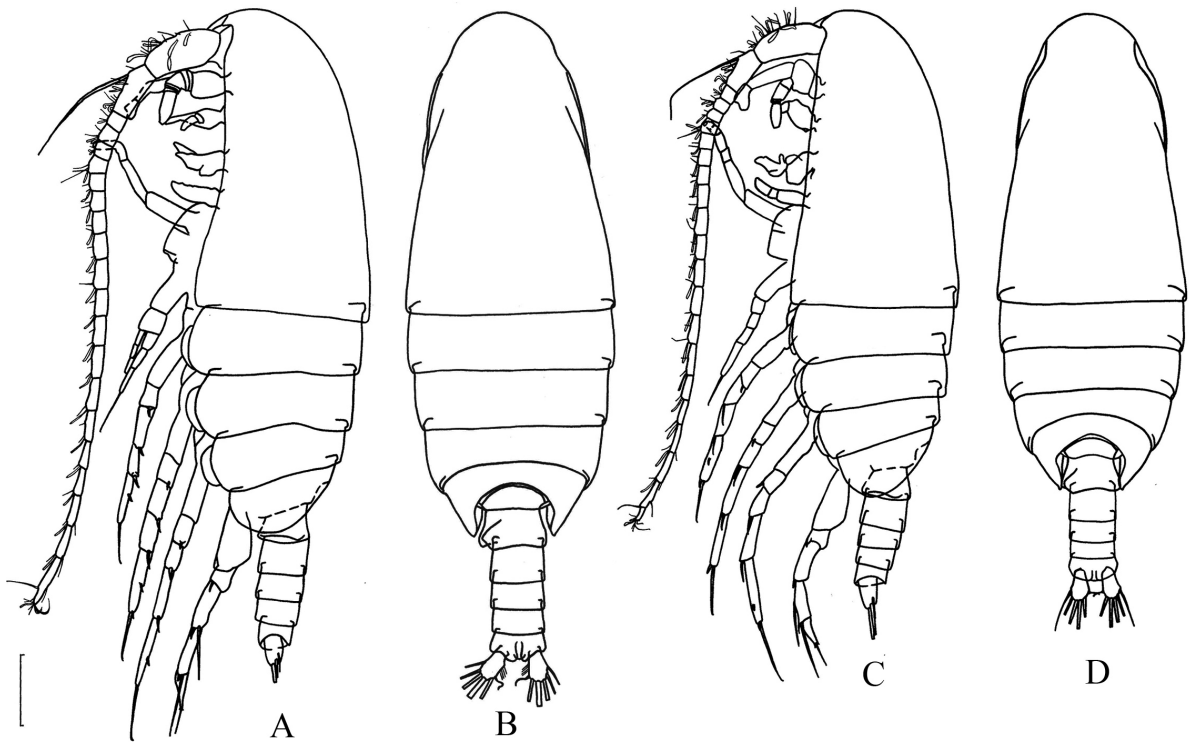

Fig. 2. Habitus of *Nannocalanus major* (A, B) and *N. sewelli* (C, B) males: left view (A, C), dorsal view (B, C).
Рис. 2. Внешний вид самцов *Nannocalanus major* (A, B) и *N. sewelli* (C, D): вид слева (A, C), вид со спины (B, D).

and one small seta at the base of the first pair of denticles (n = 8). The number of denticles on the inner surface of B1P5 is 10−18 (average 13.0, n=18) for the left coxopodite, and 9−15 (average 12.0) for the right one. The ratio of the serrated part to the entire part of B1P5 is 83–94% (average 90%, n=18).

In the distal part of the first segment of the abdomen (left view), both pores are located at different levels relative to its distal edge. Dorsally, only one of the pores is visible, located approximately in the middle of this segment and always above the chitinous fold. In the second segment of the abdomen (left view), the pores in its middle part are located at different levels relative to the distal edge of the segment.

Discussion

For a long time the genus *Nannocalanus* was considered monotypic. It was due to the fact that females and males from different parts of the World Ocean are morphologically poorly distinguishable. However, detailed morphological studies of populations from the Indian and Pacific Oceans have led the genus currently including four species: *N. minor*, *N. major*, *N. sewelli*

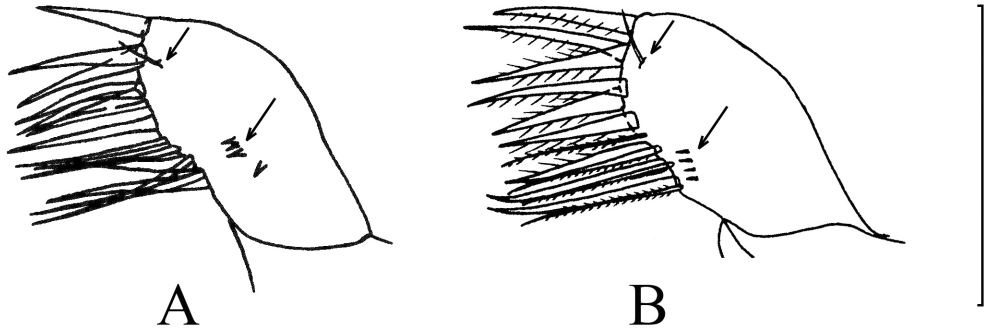

Fig. 3. The gnathobase of maxillular praecoxopodite of *Nannocalanus major* (A) and *N. minor* (B)
Рис. 3. Гнатобаза прекоксоподита максиллуллы *Nannocalanus major* (A) и *N. minor* (B).

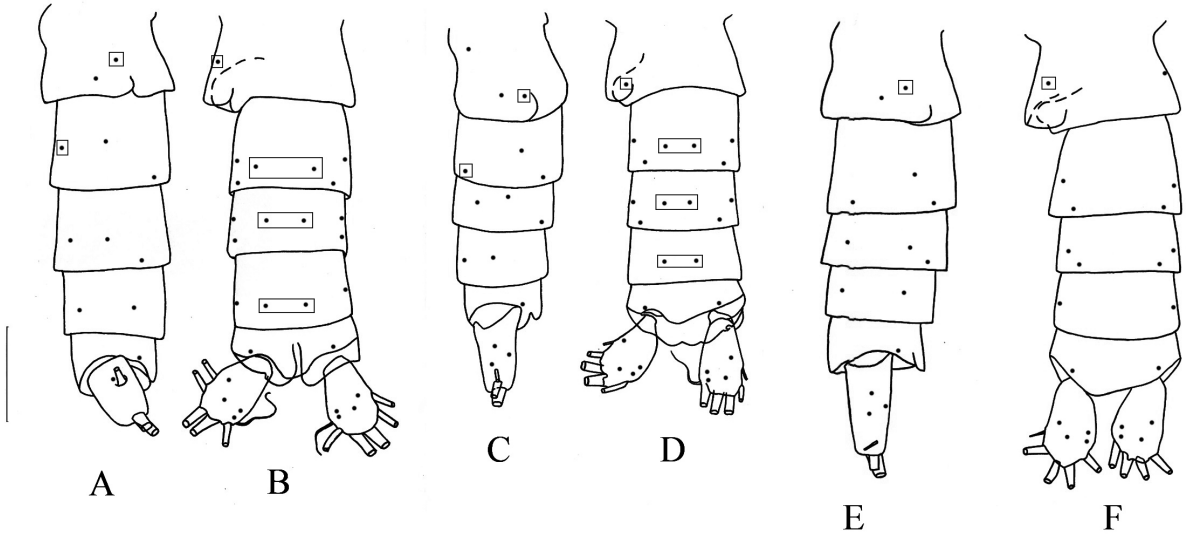

Fig. 4. Abdomen and caudal rami, left lateral view (A, C, E) and dorsal side (B, D, F) of *Nannocalanus major* (A−B), *N. sewelli* (C−D) and *N. minor* (E−F) males. Key diagnostic pores are marked with frames.

Рис. 4. Абдомен и фуркальные ветви латерально слева (A, C, E) и с дорсальной стороны (B, D, F) самцов *Nannocalanus major* (A−B), *N. sewelli* (C−D) и *N. minor* (E−F). Ключевые диагностические поры выделены рамками.

and *N. elegans* [Claus, 1863; Sewell, 1929; Kazus, 2009; Andronov, 2001]. Therefore, finding reliable diagnostic criteria became necessary since an accurate identification of these species is very important for ecological and biogeographic studies, and simple morphological methods, as our work has shown, are not always sufficient. Our fast and accessible method of integumental pore analysis solves this problem for males, allowing us to accurately distinguish the Indo-Pacific species (*N. major* and *N. sewelli*) from each other and distinguish them from the Mediterranean *N. minor*.

Morphological studies of body length, the number of denticles on the inner edge of the left and right B1P5 and the ratio of the length of the serrated part of the inner edge of the left B1P5 to the entire length conducted for *N. major* and *N. sewelli* males did not reveal significant differences between them. Denticles on the gnathobase of the maxillular praecoxopodite were present in males of all three species, however in *N. sewelli* males they were sometimes absent.

Thus, the specific pore location on the abdomen of males is the only reliable criterion for species identification of *Nannocalanus* males which can be used for further comparative studies with populations from other regions of the World Ocean.

Compliance with ethical standards

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

Ethical approval: No ethical issues were raised during our research.

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