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ONTOGENETIC DEVELOPMENT OF OLFACTORY ORGANS IN GRASS SHRIMP, *PANDALUS KESSLERI* (DECAPODA, PANDALIDAE)

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Ontogenetic Development of Olfactory Organs in Grass Shrimp *Pandalus kessleri* (Decapoda, Pandalidae). Blinova N. K., Cherkashin S. A. — The olfactory organ in decapod crustaceans is represented by the outer antennular flagellum with specialized sensilla called aesthetascs. Characteristic structures of the lateral antennular flagellum with olfactory setae in larvae, juveniles and adults of grass shrimp *Pandalus kessleri* Czerniavski, 1878 are described.

Key words: grass shrimp, olfactory organ, development, aesthetasc.

Развитие органов обоняния травяной креветки, *Pandalus kessleri* (Decapoda, Pandalidae), в онтогенезе. Блинова Н. К., Черкашин С. А. — Орган обоняния десятиногих ракообразных представлен наружными жгутами антеннул, с расположенными на них специализированными чувствительными щетинками — эстетасками. Описаны особенности строения латерального жгута антеннулы с обонятельными щетинками у личинок, молоди и половозрелых особей травяной креветки — *Pandalus kessleri* Czerniavski, 1878.

Ключевые слова: травяная креветка, орган обоняния, развитие, эстетаск.

Introduction

Today, the crustaceans olfactory system is the subject of close morpho-functional studies (McClintock et al., 2006). In decapod crustaceans, this organ is studied to a greater extent. The large body size and accessibility of chemosensor organs in such species as Caribbean spiny lobster *Panulirus argus*, California spiny lobster *Panulirus interruptus*, and American lobster *Homarus americanus* allowed their successful use in morphological and neurobiological experiments (McClintock et al., 2006; Schmidt et al., 2006; Derby, Sorensen, 2008).

The olfactory organ in decapod crustaceans is represented as the outer antennular flagellum with specialized sensilla called aesthetascs. There are lots of information on aesthetascs morphology in many Decapoda species (Ghiradella et al., 1868; Snow, 1973; Tierney et al., 1986; Blinova, 1988; Steullet et al., 2000). Characteristic structures of olfactory receptors in grass shrimp *Pandalus kessleri* Czerniavski, 1878 has been already described (Blinova, 1988, 2008). However, data on the ontogenetic development of chemosensor structures in marine decapod crustaceans are still scanty.

The aim of the study was description of characteristic structures of olfactory receptors in grass shrimp *P. kessleri* at different stages of individual development.

Material and methods

Outer structure of antennules with olfactory setae was studied in larvae of grass shrimp *P. kessleri* grown in laboratory (Blinova, Cherkashin, 1999). Juveniles and adults for investigations catches in the Bay of Peter the Great in the Sea of Japan.

Morphometric parameters were studied with using of the scale ruler in MBS−9 binocular and Biola microscope with ocular micrometer. The following parameters were measured: body length, length of the outer antennular flagellum, length of aesthetasc, diameter of proximal and distal parts of antennular flagellum. Body length was measured from the beginning of rostrum till the end of telson; total length of antennular flagellums — from the base of distal annulet; diameter of the distal part of flagellum — at the level of fine appendage without aesthetascs.

For the light microscopy, total preparations of the outer flagellum of antennulae stained with methylene blue were used. For scanning electron microscopy, pieces of antennular tissue were fixed in 5% glutaraldehyde in cacodylate buffer at pH 7.4 for 1.5 hours. Post fixation was performed in 1% of OsO_4 solution in cacodylate buffer at 4°C for 1 hour. Dehydration was carried out in alcohols of increasing concentrations, material was dried at the critical point, covered with gold and examined under scanning microscope JSM 25S ("JEOL", Japan).

In every size-age group, from 5 to 10 specimens were studied. Totally, 80 animals were studied. Morphometric data of the size-age groups with the number of specimens more than 5 are given with arithmetic mean of error. For existing set of values, with using of MS Excel software, regression equation, significance level of approximation (R^2) and Pearson's correlation coefficient (r) for linear function are calculated.

Results

Outer structure of antennules with olfactory setae was studied in the early ontogenesis of *P. kessleri* at 4 stages (after Kurata, 1955) of larval development. The transition from one stage to another is associated with moulting. Duration of the period between moults was 4−5 days.

Body length of larvae grown under laboratory conditions was 9 mm on average. Antennule unsegmented, its distal part is divided into two branches which further give rise to internal and external flagellums (fig. 1). Internal branch is a short feathery seta, 260 μm long, sitting on a circular prominence. The length of external branch significantly exceeds that of the inner (650 μm) due to a fine distal process, and on the ventral side it has two smooth setae 120 μm in length each. Morphometric data on antennules and aesthetascs in grass shrimp are presented in table 1.

The average size of the second stage larvae was 10.5 mm. Antennule has annuleted structure: stem of three annuli and formed inner and outer flagellums. Outer flagellum is divided into three annuli bearing 12 aesthetascs on the ventral side (fig. 2).

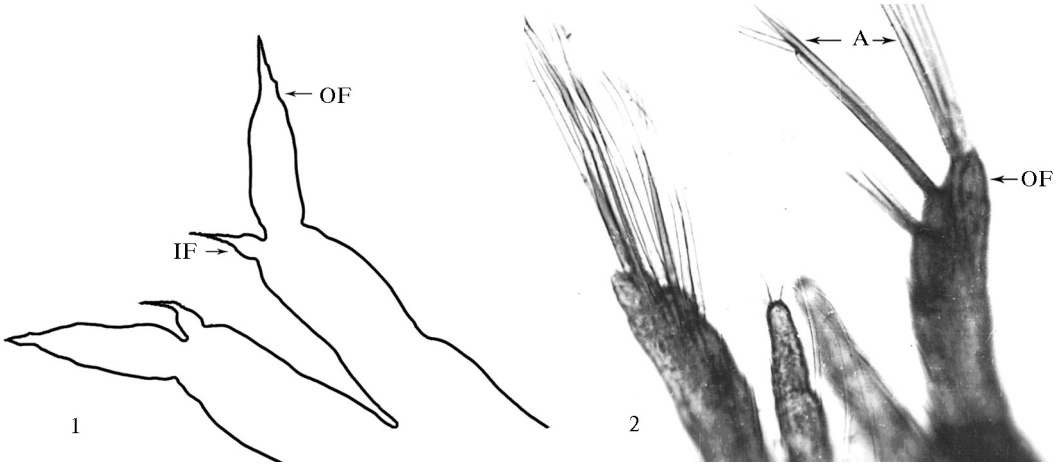

Fig. 1. Antennules of *Pandalus kessleri* at the I larval stage: OF — outer flagellum; IF — inner flagellum. Magnification x30.

Рис. 1. Антеннулы *Pandalus kessleri* на I стадии личиночного развития: OF — наружный жгут; IF — внутренний жгут. Увеличение x30.

Fig. 2. Distal part of antennules of *Pandalus kessleri* at the II larval stage: OF — outer flagellum; A — aesthetascs. Magnification x63.

Рис. 2. Дистальная часть антеннулы *Pandalus kessleri* на II стадии личиночного развития: OF — наружный жгут; A — эстетаски. Увеличение x63.

Table 1. Morfometric indices of the olfactory organs in larvae, juveniles and adults of *Pandalus kessleri*

Таблица 1. Морфометрические параметры органов обоняния у личинок, ювенильных и половозрелых особей *Pandalus kessleri*

Body length, mm	Length of outer flagellum, mm	Number of flagellum's annuli, psc	Diameter of flagellum's base, mm	Diameter of the peak, mm	Aesthetasc length, mm
		Larvae			
10.5	0.35	3	0.11	—	0.35
12.0	0.46	3	—	—	0.40
13.0	1.05	4	—	—	0.42
		Juveniles			
16 ± 0.70	1.5 ± 0.06	—	0.15 ± 0.006	0.096 ± 0.00	0.45 ± 0.020
18 ± 0.02	1.7 ± 0.07	—	0.17 ± 0.009	0.096 ± 0.03	0.47 ± 0.020
37 ± 0.30	5.5 ± 0.20	27 ± 0.4	0.28 ± 0.040	0.130 ± 0.010	0.48 ± 0.018
		Adults			
55 ± 1.8	8.5 ± 0.58	40 ± 1.1	0.41 ± 0.03	0.31 ± 0.03	0.65 ± 0.060
86 ± 2.2	16.3 ± 0.90	65 ± 0.3	0.61 ± 0.03	0.39 ± 0.02	0.65 ± 0.014
110 ± 1.8	18.1 ± 0.31	82 ± 0.6	0.70 ± 0.01	0.39 ± 0.03	0.68 ± 0.016
131 ± 2.1	19.6 ± 0.50	85 ± 1.3	0.74 ± 0.06	0.39 ± 0.02	0.78 ± 0.050

Larval aesthetascs are fine, smooth setae 350 μm in length with thickened base and annulated rod. On flagellum, they form transverse rows, 3 hairs in each. One row of olfactory setae is located on the distal annulet of the flagellum, two other rows are on the second annulet and one row of growing aesthetascs (length 50–60 μm) on the proximal annulet. Internal antennular flagellum is smaller than the outer one and consists of two annuli (table 1).

At stage III of larval development, number of annuli on shrimp internal antennulae flagellum increases, as well as all morphometric parameters (body length 12 mm). However, the number of annuli of the outer flagellum and the number of aesthetascs on them remain unchanged. The length of aesthetascs reaches 400 μm. Internal antennulae flagellum of the IV stage larvae consists of six annuli. It equalizes in length with the outer flagellum and even becomes larger. Short projection of the distal part of the outer flagellum is isolated in a separate annulet, and the 5th group of growing aesthetascs appears on proximal annulet. By the end of larval development, antennulae of grass shrimp have 15 olfactory setae.

Statistical analysis of morphometric data indicated an exponential (for the length of the outer flagellum, number of annuli) and power (for aesthetascs) dependence on larval body length (table 2).

Table 2. Dependence of morphometric parameters of olfactory organ on body length of *Pandalus kessleri* at different ontogenetic stages

Таблица 2. Зависимость морфометрических параметров органа обоняния от длины тела *Pandalus kessleri* на различных этапах онтогенеза

Length of outer flagellum.	Number of flagellum's annuli	Diameter of flagellum's foundation	Diameter of the peak	Aesthetasc length
		Larvae		
$Y = e\ 0.549\ x$ ($R^2 = 0.92$)	$Y = 2.47\ e\ 0.143x$ ($R^2 = 0.75$)	—	—	$Y = 0.351X\ 0.168$ ($R^2 = 0.98$)
		Juveniles		
$Y = 2X - 1.1$ ($R^2 = 0.78$) $r = 0.99$	-	$Y = 0.065X + 0.07$ ($R^2 = 0.86$) $r = 0.99$	$Y = 0.017X+0.73$ ($R^2 = 0.75$) $r = 0.99$	$Y = 0.450X\ 0.059$ ($R^2 = 0.99$)
		Adults		
$Y = 3.51X+6.85$ ($R^2 = 0.84$) $r = 0.95$	$Y = 15.2X+30$ ($R^2 = 0.90$) $r = 0.97$	$Y = 0.108X+0.35$ ($R^2 = 0.89$) $r = 0.97$	–	$Y = 0.237X-0.06$ ($R^2 = 0.73$) $r = 0.94$

Note. R^2 — significance level of approximation; r — Pearson's correlation coefficient.

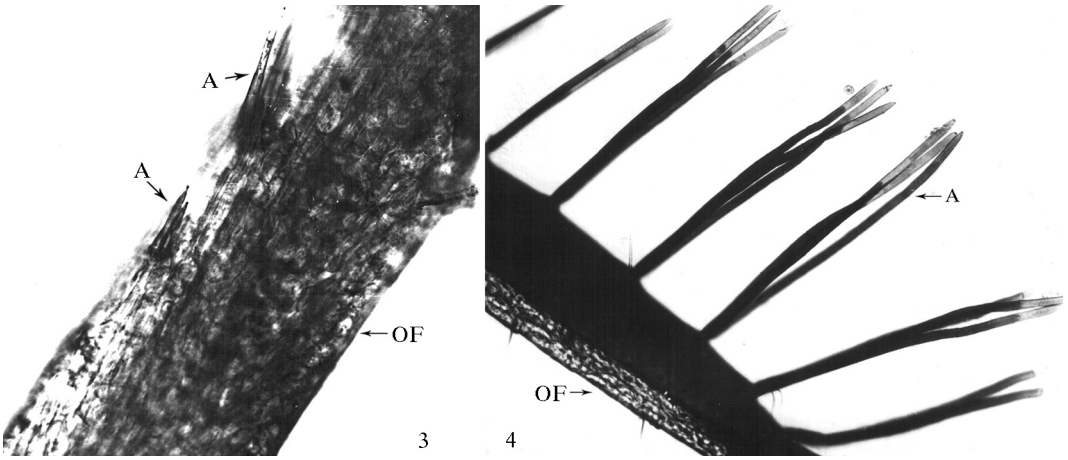

Fig. 3. New aesthetascs on proximal annuli of outer antennular flagellum of *Pandalus kessleri*: OF — outer flagellum; A — aesthetascs. Magnification x160.

Рис. 3. Растущие эстетаски на проксимальных члениках наружного жгута антеннулы *Pandalus kessleri*: OF — наружный жгут; A — эстетаски. Увеличение x160.

Fig. 4. Outer antennular flagellum with aesthetascs of juvenile *Pandalus kessleri*: OF — outer flagellum; A — aesthetascs. Magnification x63.

Рис. 4. Наружный жгут антеннулы с эстетсками у ювенильных особей *Pandalus Kessler*: OF — наружный жгут; A — эстетаски. Увеличение x63.

After the next moulting, number of aesthetascs increases in proportion to size of animal's body. On outer antennular flagellum of juvenile shrimps, thin distal part which bears no olfactory setae is formed. In the proximal part of the flagellum, zone with increased number of growing aesthetascs becomes visible (fig. 3). On annuli of the middle part of the outer flagellum, aesthetascs are ordered in rows of 3 setae each (fig. 4). For determined morphometric parameters, linear dependence on the length of animal's body was noted (table 2). With increased body length, the number of annuli and the length of outer antennular flagellum increases in direct proportion: Pearson's coefficient of linear correlation is rather higher, 0.99. The dependence of every morphometric parameter of outer antennular flagellum (Y) on body length (X) of young and adult animals represented linear function. However, the length of the aesthetascs in the young is increasing as well as in the larvae, according to power function. The number of aesthetascs per annulet of the flagellum remains relatively unchanged. Regression equation reflecting the dependence of morphometric parameters of olfactory organs on shrimp's body length are given in table 2.

Antennules of mature specimens of grass shrimp *P. kessleri* consisted of foundation divided into three parts, interior and outer flagellum (Blinova, 1988; fig. 1). Outer antennular flagellum is slightly shorter than inner one. In grass shrimps of the size 131 mm in length, antennulae is 37 mm, the length of the outer flagellum — 19.6 mm. Normally, outer flagellum consisted of 85 annuli. At the level of proximal annuli, its diameter is 740 μm, and at the level of 60–70 annuli (in place of the transition to a thin distal) is reduced to 390 μm.

Outer antennular flagellum is flattened dorso-ventrally (fig. 5). It has considerable longitudinal groove on the ventral side where aesthetascs are located. This groove is non-uniform, it is pronounced more on the medial side and in the middle of antennulae. As a result, the groove has two limiting lateral lobes: larger medial and smaller lateral. On approaching the thin distal part of antennulae, groove is evened out. Cuticle inside the groove has numerous folds. Each annulet in the middle part of antennulae (excluding 8–10 proximal and 15–20 distal) has two transverse rows of aesthetascs. One

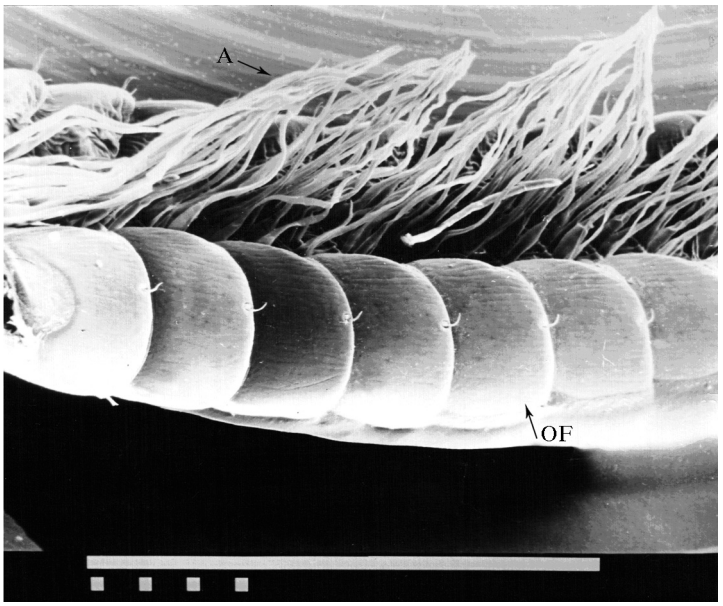

Fig. 5. Outer antennular flagellum of antennule of *Pandalus kessleri* with aesthetascs: OF — outer flagellum; A — aesthetascs. Scanning electronogramme. Scale bar 1000 μm.

Рис. 5. Наружный жгут антеннулы *Pandalus kessleri* с эстетасками: OF — наружный жгут; A — эстетаски. Сканирующая электронограмма. Масштабная линейка 1000 мкм.

row consists of 6 sensory hairs. Proximal 6–8 annuli of the flagellum have no aesthetascs. Each next annulet contains two rows of 3, then 4, 5, and at last of 6 olfactory setae.

Aesthetasc of mature grass shrimp is a fine hairless villus embedded into the cuticular fold of antennulae. It is formed by expanded ampoule shaped base and a rod of segmented structure. In specimens with average body length of 131 mm, the total length of hairs in the middle part of antennulae is 780 μm, and the hairs located on the distal and proximal annuli — 620 μm. Diameter of hairs at the base is 30 μm. The height of the rod annuli increases from 20 mm at the base to 40 μm in the middle. The apical part of the aesthetascs is characterized by thin seta, 30–35 μm in length. In cuticular fold of antennulae, small pores (10 pores in each hair) are located proximally from aesthetasc.

In some specimens of *P. kessleri* with body length of 130–140 mm, special fan-shaped setae were found behind aesthetascs. These setae have rounded base and up to 40 fused elongated «petals», the total size of which does not exceed 35 μm.

Discussion

Distant perception of chemical stimuli in crustaceans by antennular chemoreceptors (Antenna 1) is studied. However, antennule of crustaceans is multifunctional sensory organ with numerous sensitive structures of different modalities. Among sensory setae of outer antennular flagellum in grass shrimp, as well as in other species of decapod crustaceans, the most numerous specific olfactory setae are aesthetascs.

Sensory setae appeared on antennules on early ontogenetic stages. Grass shrimp has incomplete metamorphosis — hatched larvae look like adults. Nevertheless, morphological studies of olfactory organs on various ontogenetic stages *P. kessleri* revealed significant differences between the outer antennular structure and olfactory setae in larvae, juveniles and adults. During the growth and development of grass shrimp, the structure of olfactory organs is being improved and complicated to meet increasing sensory needs of the animal.

The outer antennular structure with their sensory setae in *P. kessleri* larvae has been described in detail earlier (Blinova, Cherkashin, 1999). Hatched larvae of grass shrimp have two smooth setae on each ventral outer antennular branch. These setae may be precursors of aesthetascs. Before the first moult, their outer structure is not similar to that of aesthetascs. The larvae of some Far Eastern species of shrimps on the early stages of development also have sensory setae on their antennules (Ivanov, 1971). In ontogenesis, aesthetascs in *P. kessleri* appear on the II stage of larval development: 9 morphologically formed setae and 3 young growing setae (Blinova, Cherkashin, 1999). Kurata (1955) also noted that in this period outer antennular flagellum in grass shrimp consists of three annuli with four groups of sense hairs. By the end of larval development, the outer antennular flagellum totally contains 15 aesthetascs. In young animals, newly emerged setae starts functioning after a long time. It is known that in the post-embryonic period larvae of crustaceans complete their organogenesis (Ivanova-Cazas, 1979).

The growth and formation of aesthetascs in the young individuals starts in the proximal part of the outer antennular flagellum and then wave of turnover goes distally to fine prominence where aesthetascs destructed. On the outer antennular flagellum of grass shrimp, as well as other decapod crustaceans, from proximal to distal parts, the following zones are seen: proliferation where setae are formed; zone with functionally mature aesthetascs and, finally, senescense zone (Schmidt et al., 2006). Sometimes the transition zone is distinguished between the proliferation zone and zone with mature aesthetascs (McClintock et al., 2006). It covers proximal annuli of the outer flagellum with growing aesthetascs. For *P. kessleri,* the annuli with functionally mature sensory setae form most part of the outer antennular flagellum (50%). The lifetime of newly appeared setae in juvenile lobster *P. argus* is not associated with moulting and this is a period of approximately 3 to 6 moults (Steullet et al., 2000). Differentiation and functional formation of olfactory sensory neurons in aesthetascs occurs over rather long period of time: from several weeks to several months. Flagellar growth due to increasing number of annuli in the growth zone with a constant number of hairs on each annulet of the middle part of antennulae enables the animal to maintain the constancy of the receptive field and sensitivity to chemicals.

In ontogenesis, in the middle part of the outer antennular flagellum, the number of aesthetascs in one row increases from three to six. In adults, protective and promoting perception of chemical stimuli structures and appliances appear. Deepening of aesthetascs in *P. kessleri* into the groove with two lateral lobes prevent damaging of setae. For this purpose, lobster *P. argus* has powerful guard bristles forming two longitudinal rows medially and laterally from aesthetascs (Derby et al., 2001). Possible functions of pores at the foundation of olfactory setae are associated with release of a secret from so-called "aesthetasc tegumental glands" preventing aesthetascs friction and contamination (Schmidt et al., 2006). Fan-shaped setae revealed behind the aesthetascs foundation in some large specimens of grass shrimp are probably a sign of sexual dimorphism and participate in the reception of pheromones in females (Blinova, 2008). Fine apical villus of distal part of setae rod may increase probability of interaction with olfactory stimulus.

Morphometric parameters of the olfactory organ larvae has nonlinear, but exponential or power dependence on the total length of the grass shrimp body. The power function was proposed to determine dimensions of different parts of body in *P. kessleri* larvae at known carapace length (Kurata, 1955). In contrast to obtained power dependence between larval body length and antennular morphometric parameters, young and adult animals have mainly simple linear dependence. Changing the nature of morphometric data of olfactory organs from the body length during ontogenesis is likely due to the fact that the development of the peripheral part of olfactory system is the most

intensive during the larval period and completes in mature animals. Based on these data, we can assume that during the larval and juvenile stages of development of the grass shrimp, morphological basis for functioning of olfactory organ and related innate behavioural reactions are formed.

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