RECONSIDERATION OF ONTOGENETIC CHANGES OF THE MAXILLULA IN THE SPECIES OF GENUS *LOXOCONCHA* SARS, 1866 (CRUSTACEA, OSTRACODA, PODOCOPIDA)

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ABSTRACT

Ostracod maxillula is the fourth head appendage and it is the one of key indicators in taxonomy, phylogeny and evolution. So far, some reports on ontogenetic changes of the maxillula of the genus *Loxoconcha* have been already published. In previous study, it was shown that the general development and number of setae on the maxillula are identical between the two species (*L. noharai* Le & Tsukagoshi, 2014 and *L. japonica* Ishizaki, 1968) of this genus from the second (A-7) to the fourth instars (A-5), and the differences between the two species appear in and after the fifth instar (A-4). In the present report, by observation of the maxillulan ontogenetic changes in *Loxoconcha sesokoensis* Le & Tsukagoshi, 2014, the differences in the general development and number of setae among three *Loxoconcha* species (i.e., *L. noharai* Le & Tsukagoshi, 2014; *L. japonica* Ishizaki, 1968 and *L. sesokoensis* Le & Tsukagoshi, 2014) can be found at the earlier instar, the fourth instar (A-5). This finding contributes to the more accurate understanding of ontogenetic changes of maxillula and the phylogeny in the genus *Loxoconcha.*

Keywords: Loxoconcha, chaetotaxy, maxillula, taxonomy, phylogeny.

1. INTRODUCTION

The genus *Loxoconcha* was established by Sars in 1866 [1]. It is one of the most diverse recent ostracod genera. Species of this genus are widely distributed in low-to-middle-latitude areas of marine and brackish waters and up to more than 150 living and 350 fossil species have been identified in the world [2]. The history of this genus is being debated. So far, this genus is considered to originate in the late Palaeogene and started its adaptive radiation in the Neogene [3]. The oldest record of the genus *Loxoconcha* in the World have been found in Indo-West Pacific Region in the Oligocene (possibly the Late Eocene) [4-6].

The maxillula is one of the common parts of crustacea. For the case of ostracods, the maxillula is the fourth head appendage. It lies immediately behind the mandibles and has two functions, feeding and, in some groups, respiration [7]. In general structure of podocopan ostracods, the maxillula consists of a protopodite, bearing antero-medially an endopodite (commonly referred to as a palp; often segmented, lying parallel to the three endites) and three endites (sometimes referred to as masticatory lobes), all of which terminate in several short setae [7]. The endites and palp assist the mandibles in moving food towards the mouth and removing waste particles from the mouth region. The maxillula also consists of an extremely well developed epipodite branchial plate with radiating long, setulous, or feathery setae posteriorly and several reflexed setae point forwards [7]. The branchial plate beats

continously, circulating water within the body cavity and presumably assisting with respiration. The primary function of the current produced by the branchial plate is to maintain a flow of oxygenated water through the domicilium [8, 9].

Presently, published illustrations of maxillula of the *Loxoconcha* species at the adult stage as well as ontogenetic instars are fewer than those of other appendages, e.g., carapace, attennula, attenna… probably because the maxillula is very fragile and is normally recognized that it has little perceived taxonomic meaning (e.g., comparing with copulatory organ). The maxillulla is only well considered in the majority of species, has similar morphology and has the same function in all groups, and is not sexually dimorphic or involved in reproduction. As for the maxillulan ontogeny of *Loxoconcha*, only Smith and Kamiya (2003), Le *et al.* (2016) could be referred [10, 11]. Following them, maxillula appears in the instar A-7 as anlage stage. Anlage consists of simple elongated protrusion from body. In the instar A-6, the morphology of maxillula is rather simple and the same about maxillular structure among species. By observation of maxillular ontogenetic changes of *L. sesokoensis*, the aim of this paper was to document the number of setae on the different parts of ontogenetic maxillula of three *Loxoconcha* species, and to seek to identify phylogenetically significant trends.

2. MATERIALS AND METHODS

2.1. Sampling and specimen preservation

Superficial sediments were collected from Sesoko island, the Okinawa Islands, Southern Japan in December, 2015 (Fig.1). Sampling was conducted during low tide in the study areas. At the sampling points, where the water depth was less than 30 cm, the uppermost 5 mm of the active layer of sediment was scooped into a plastic bottle using a scoop (a flat scoop with dimensions of 12 cm × 15 cm or a rectangular scoop of 4 cm × 7 cm, depending on the degree of surface irregularity). Then, all of the collected specimens were fixed in 5–10% formaldehyde that had been neutralised with hexamethylenetetramine, before being washed through 16-mesh (# 1 mm) and 250-mesh (# 0.063 mm) sieves. Part of the washed material was fixed with 70–80% alcohol for later observations of the appendages, and the remaining material was dried.

2.2. Specimen treatment

About 50 specimens of *L. sesokoensis* were used in this study. The specimens were dissected under a binocular microscope in the laboratory. Their appendages and carapaces were then observed and sketched using a differential interference contrast microscope with a camera lucida (BX-50, OLYMPUS) to obtain illustration photos. Also for the dissected specimens, soft parts were mounted on a slide glass in the "Neo Sigaral" agent and carapaces were on a cardboard slide with single hole. At the same time, the number of setae on maxillula (three endites, endopodite, outer 1st podomere of endopodite, exopodite) (Fig. 2) was counted and the chaetotaxy of maxillula was also observed. The dimensions of the valves were measured using some computer software such as ImageJ, Adobe Photoshop, and Paint as well as the microscope.

Dried carapaces and individuals were coated with gold using a quick auto-coater (JFC-1500, Ion Sputtering Device). They were then observed with a scanning electron microscope (JSM-5600LV, JEOL). Scanning electron microscope photos were subsequently used for identification of carapace sizes, pore groups of all ontogenetic stages (Fig. 3 and Table 1).

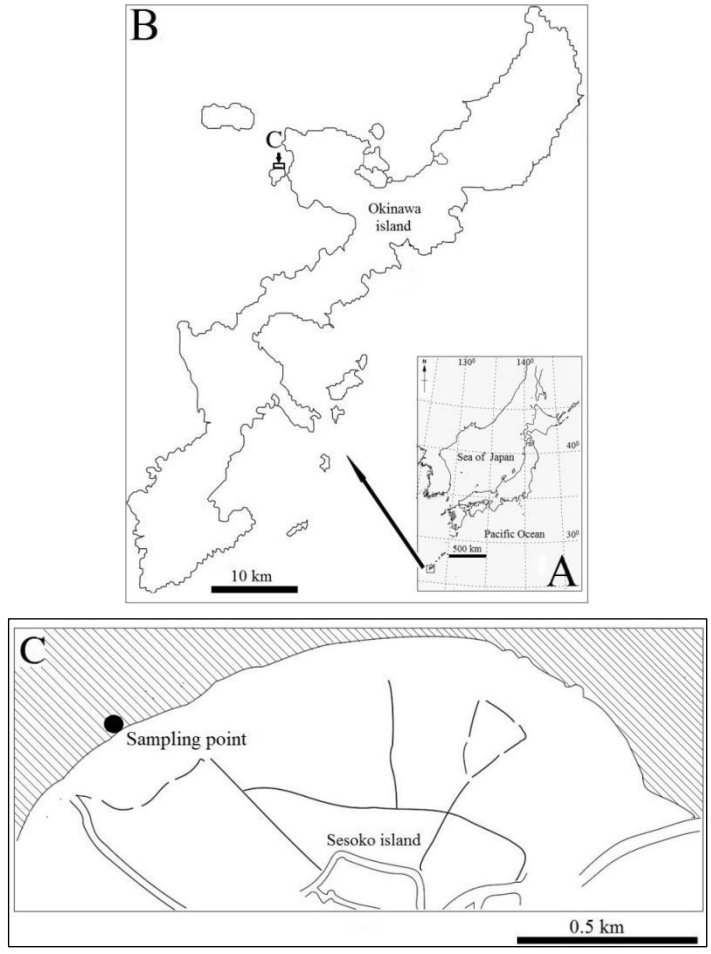

Figure 1. Maps showing location of the study area. A: Map of Japan;
B: Map of the Okinawa Islands; C: Map of Sesoko Island showing sampling site.

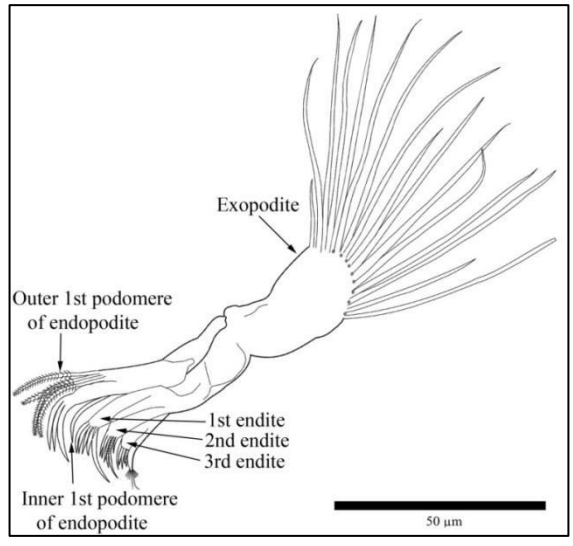

Figure 2. Sketching of adult maxillula of *Loxoconcha sesokoensis* Le & Tsukagoshi, 2014 [12]

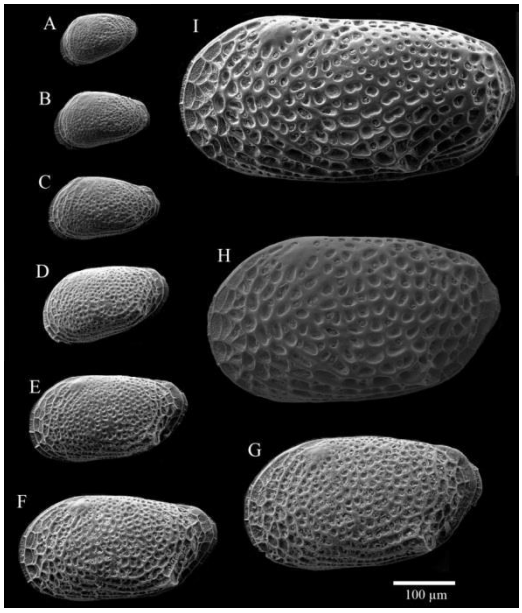

Figure 3. Left carapace in external lateral view of *Loxoconcha sesokoensis* through ontogeny. A. Instar A-7; B. Instar A-6; C. Instar A-5; D. Instar A-4; E. Instar A-3; F. Instar A-2; G. female of instar A-1; H. female of adult; I. male of adult.

3. RESULTS

3.1. Dimension of the valves of three *Loxoconcha* species following the each instar

For each species, dimensions of carapaces continuously increase through ontogeny from the instar A-7 to adult. It means that the older the individual is, the bigger it is. Comparison of the three *Loxoconcha* species indicates the size of carapaces of *L. japonica* is bigger than that of the other two species, i.e., *L. noharai* and *L. sesokoensis* at all instars (Table 1). However, carapaces of *L. sesokoensis* seem to be somewhat larger than *L. noharai* at the instars A-7 and A-6, but this trend is converse at the instars from A-5 to adult. Basically, the larger carapace is the higher the number of setae of the maxillula gets (Tables 1, 2).

Table 1. Dimensions of carapaces of the instars of three *Loxoconcha* species

Species / Instar	*L. sesokoensis*			*L. noharai*			*L. japonica*	
	Height (µm)	Length (µm)	n	Height (µm)	Length (µm)	n	Height (µm)	Length (µm)
A-7	70.8	121.0	2	70.8	116.5	4	84.0	131.0
A-6	96.6	142.8	5	94.3	137.5	4	99.0	160.5
A-5	101.4	163.4	14	104.0	172.3	4	116.5	187.5
A-4	122.8	194.3	4	120.0	201.0	4	141.5	233.5
A-3	143.0	244.4	2	148.5	246.2	12	175.5	290.5
A-2	168.7	293.5	6	183.3	307.3	4	224.0	380.0
A-1 (Female)	202.0	345.5	2	230.0	380.0	7	292.5	474.0
A-1 (Male)	210.0	361.6	3	237.8	405.3	6		
Adults (Female)	250.0	405.0	3	284.6	462.3	8	396.0	573.5
Adults (Male)	255.0	475.0	3	281.0	513.0	8		
Total			41			61		

Note: Data of *L. noharai* is taken from [11]; of *L. japonica* from [10].

3.2. The number of setae of maxillula of *Loxoconcha sesokoensis* following the different instars

In *Loxoconcha sesokoensis*, the maxillula first appears at the instar A-7 as an anlage state, with a simple structure (Table 2). Maxillular structure of this instar is very simple, not clear and tiny. Then, the maxillula starts to develop at the instar A-6 with several setae at the three endites and exopodite, but no seta at endopodite, outer 1st podomere of endopodite and inner 1st podomere of endopodite. From the instar A-5 (Fig. 4B and Table 2), the maxillula develops with the setae locating at all parts of maxillula. Hence, the number of setae of each different part of maxillula in *L. sesokoensis* continuously increases through the stages of ontogeny and reaches to the maximum at pre-adult stages (Table 2), e.g., the number of setae of three endites gets the peak at the instar A-2, of endopodite at the instar A-3, of exopodite at the instar A-1, of outer 1st podomere of endopodite at the instar A-1 and of inner 1st podomere of endopodite at the A-5.

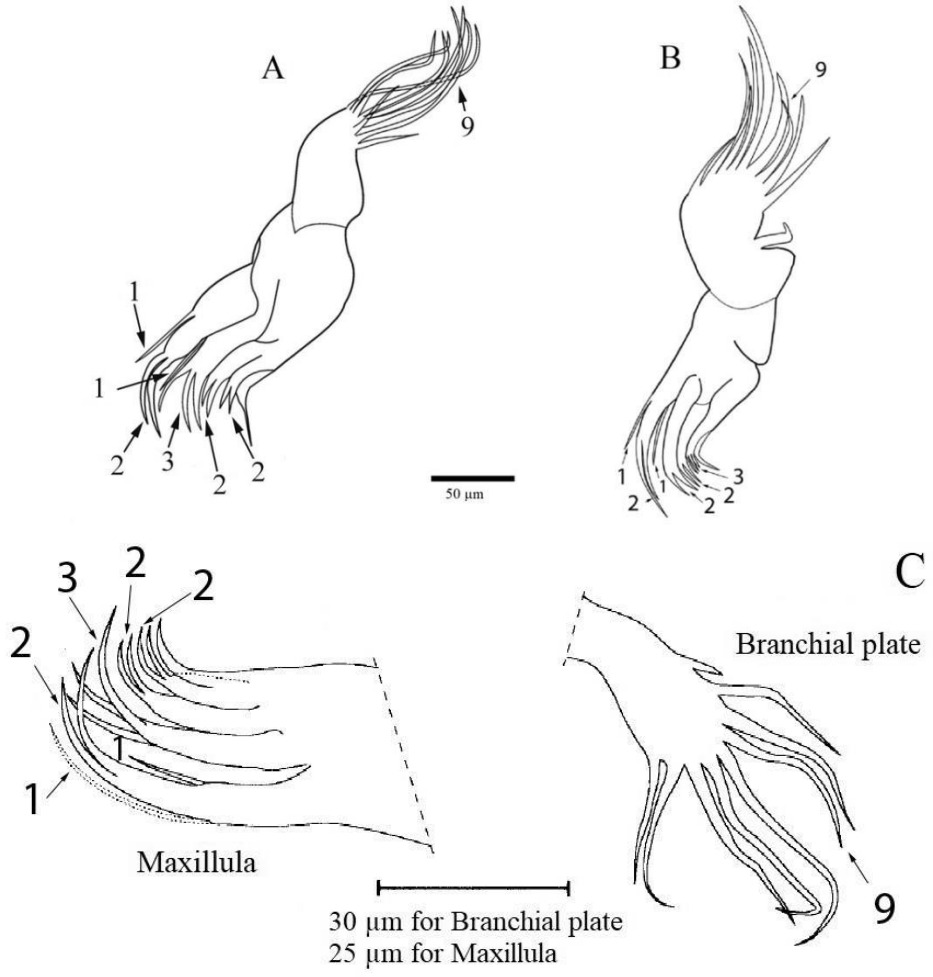

Figure 4. Sketching of maxillula of the instar A-5. A: *Loxoconcha noharai* Le & Tsukagoshi, 2014 [11]; B: *L. sesokoensis* Le & Tsukagoshi, 2014; C: *L. japonica* Ishizaki, 1968 [10].
Figures indicate the number of setae on different parts of maxillula.

Table 2. The development of number of setae on maxillula of three species of the genus *Loxoconcha*

Species	Parts of the maxillula	Molt instar (A-1, A-2, etc., in descending order of size)							
		A-7	A-6	A-5	A-4	A-3	A-2	A-1	Adult
L. noharai	Endopodite	Anlage	0	2	2	3	3	3	3
	First endite		1	3	3	3	6	6	6
	Second endite		2	2	2	3	6	6	6
	Third endite		2	2	3	3	4	4	4
	Exopodite		6	9	11	13	15	16	16
	Outer 1st podomere of endopodite		0	1	2	2	3	4	4
	Inner 1st podomere of endopodite		0	1	1	1	1	1	1
	Total		11	20	24	28	38	40	40
	n	2	5	3	4	5	4	6	2
L. sesokoensis	Endopodite	Anlage	0	2	2	3	3	3	3
	First endite		1	2	3	3	5	5	5
	Second endite		2	2	3	3	6	6	6
	Third endite		2	3	2	3	5	5	5
	Exopodite		6	9	11	13	14	15	16
	Outer 1st podomere of endopodite		0	1	2	2	3	4	4
	Inner 1st podomere of endopodite		0	1	1	1	1	1	1
	Total		11	20	24	28	37	39	40
	n	2	3	8	4	3	5	3	2
L. japonica	Endopodite	Anlage	0	2	2	3	3	3	3
	First endite		1	3	3	3	4	4	4
	Second endite		2	2	2	3	5	5	5
	Third endite		2	2	2	3	4	4	4
	Exopodite		6	9	11	11	14	15	17
	Outer 1st podomere of endopodite		0	1	2	2	2	3	3
	Inner 1st podomere of endopodite		0	1	1	1	1	1	1
	Total		11	20	23	26	33	35	37

Note: Data of *L. noharai* is taken from [11]; of *L. japonica* from [10].

4. DISCUSSION

Ontogeny of *Loxoconcha noharai* was described by Le *et al*., 2016 [11]; that of *L. japonica* by Smith & Kamiya (2003) [10]. According to these authors, the general

development and number of setae on the maxillula are identical between the two species (*L. noharai* and *L. japonica*) of this genus from the second (A-7) to the fourth instars (A-5), and the differences between the two species appear in and after the fifth instar (A-4). In this report, by observation of the maxillulan ontogenetic changes in *Loxoconcha sesokoensis* Le & Tsukagoshi, 2014, the differences in the general development and number of setae among three *Loxoconcha* species (i.e., *L. noharai* Le & Tsukagoshi, 2014; *L. japonica* Ishizaki, 1968 and *L. sesokoensis* Le & Tsukagoshi, 2014) can be found at the earlier instar, the fourth instar (A-5). At the instar A-5, the total number of setae of three endites is the same among three species, however the difference in the number of setae of each endite among three species is found. It means that the number of setae on the first, second and third endites of *L. sesokoensis* is 2, 2 and 3, respectively, meanwhile that of *L. noharai* as well as of *L. japonica* is 3, 2 and 2, respectively (Fig. 4).

From A-4 to adult stages, the numbers of setae on some parts (endopodite, three endites, exopodite and the outer first podomere of the endopodite) of the maxillula are slightly different among three species (Fig. 4). However, there are some similar developmental trends in the three species in this period (Table 2). Specifically, the number of setae on the endopodite peaks in the instar A-3 in the three species, whereas the number of setae of outer first podomere of the endopodite peaks in the instar A-1. Additionally, in the three species, the chaetotaxy of three endites remains unchanged from the instar A-2 to the adult stage. Only one seta on the inner first podomere of the endopodite on the maxillula is observed in the three species throughout the ontogeny stages, and this seta first forms in the instar A-5 (Fig. 4 and Table 2). According to Ishii *et al*. (2005) [13], all *Loxoconcha* species have the same number of pore systems on the carapaces from A-8 to A-4 instar stages, and differences in the number of pores among these species appear from the instar A-3 onwards. However, in the present study, differences in the number of setae on the maxillula were first observed in the instar A-5 (Fig. 3 and Table 2). These observations indicate that species-specific features of the sub-groups of genus *Loxoconcha* are established earlier in development for the maxillula compared to the pores on carapace (Fig. 5). These divisions of the chaetotaxy of maxillula beginning in the instar A-5 may be useful for interpreting the phylogeny of the genus *Loxoconcha*.

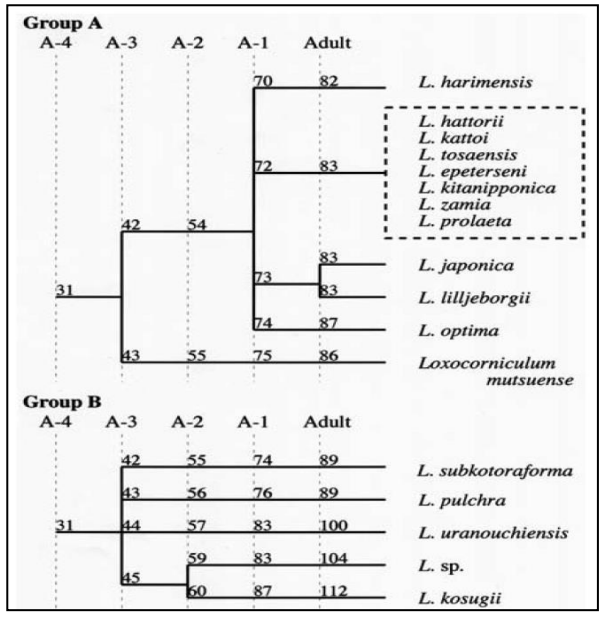

Figure 5. Difference of distributional pattern of pore system on carapace among species of the genus *Loxoconcha* [13]

5. CONCLUSION

The general development trend of maxillula in *Loxoconcha sesokoensis* is similar with *L. noharai* and *L. japonica*. In the instar A-7, a small anlage of the maxillula is formed. In the instar A-6, three endites and exopodite are formed while the endopodite is still in a basic form, undeveloped. In the instar A-5, the maxillula strongly develops to make a form similar to that of the adult. The number of setae in different parts of maxillula gradually increases through ontogeny and it gets the maximum number at the different instars.

The general development and the number of setae on the maxillula of three species, *L. sesokoensis*, *L. noharai* and *L. japonica* are completely identical each other from the second (A-7) to third instars (A-6), but different in and after the fourth instar (A-5). This can be considered as a phylogenetic relationship of the genus *Loxoconcha*.

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TÓM TẮT

XEM XÉT LẠI SỰ THAY ĐỔI THEO GIAI ĐOẠN PHÁT TRIỂN CÁ THỂ CỦA HÀM DƯỚI CÁC LOÀI TRONG GIỐNG *LOXOCONCHA* SARS, 1866 (CRUSTACEA, OSTRACODA, PODOCOPIDA)

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Hàm dưới của nhóm Giáp xác vỏ cứng (Ostracoda) là bộ phận phụ thứ tư của nhóm này và là một trong các chỉ thị quan trong phục vụ nghiên cứu về lĩnh vực phân loại, hình thành loài và tiến hóa. Cho đến nay, chỉ có một số ít nghiên cứu về sự tiến hóa cấu trúc hàm dưới của giống *Loxoconcha* theo các giai đoạn phát triển cá thể được công bố. Các báo cáo này chỉ ra xu hướng phát triển chung của hàm dưới và số lượng tua trong các bộ phận của hàm dưới là giống nhau giữa 2 loài *Loxoconcha noharai* Le & Tsukagoshi, 2014 và *L. japonica* Ishizaki, 1968 từ giai đoạn phát triển A-7 đến giai đoạn A-5, sự khác nhau bắt đầu xuất hiện ở giai đoạn A-4. Tuy nhiên, bằng việc quan sát sự phát triển của hàm dưới theo thời gian đối với loài *L. sesokoensis* Le & Tsukagoshi, 2014, nghiên cứu này cho thấy sự khác nhau về xu hướng phát triển và số lượng tua của hàm dưới giữa 3 loài thuộc giống *Loxoconcha* được phát hiện ở giai đoạn sớm hơn, bắt đầu từ giai đoạn phát triển A-5. Kết quả nghiên cứu này góp phần hiểu chính xác hơn về sự phát triển theo thời gian của hàm dưới và về xu thế phát sinh loài của giống *Loxoconcha*.

Keywords: Giống *Loxoconcha*, sắp xếp cấu trúc, hàm dưới, phân loại, phát sinh loài.