Biological characteristics and phylogeny of the genus *Volachlamys* (Bivalvia: Pectinidae) from Japan

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Abstract

For the two forms (awajichihiro and yaminonishiki) of Hirase's scallop ***Volachlamys hirasei***, in which there was disagreement on the taxonomic treatment, analytical studies were carried out in an attempt to reveal the relationship between the two forms. Further, fossil specimens of the genus ***Volachlamys*** from various Japanese strata were examined to study the genus phylogenetically, to elaborate on their evolutional history and to resolve their taxonomic treatment.

Regarding the external morphology of the two forms, there were significant differences in morphometrics between them. Relative shell height in the yaminonishiki form tended to be larger than that in the awajichihiro form. Relative shell width and shell weight in the awajichihiro form tended to be larger than that of the yaminonishiki form. The number of radial costae in the awajichihiro form tended to be greater than that of the yaminonishiki form. Further, shell coloration differed significantly between the two forms.

Regarding the internal morphology of the two forms, no visual differentiations were detected in the internal organs between the two forms. Further, seasonal changes of weight index for each organ showed very similar patterns between the two forms. Gonadosomatic index (GSI) showed high values from spring to summer, and low values from autumn to winter. In contrast, condition factor (CF) changed with inverse pattern to the GSI, being low from spring to summer, and high from autumn to winter. The other indices such as adductor muscle, gill and mantle showed similar seasonal changes with the CF pattern. It was concluded that the seasonal changes of the indices were influenced by their propagative cycles. The similarities in the internal characteristics and their seasonal changes between the two forms suggested that they are the identical reproductive populations.

Age and growth of the two forms were analyzed by using circular annuli on the shell surface as an age indicator. Seasonal changes of marginal growth rate (MGR) of the two forms suggested that annulus formation occurs once a year, from March to June; consequently, the annuli are regarded as winter rings. Relationship between shell length at age t (L_t) and that at age t+1 (L_{t+1}) corresponded well with a linear regression of Walford growth transformation in both forms. The von Bertalanffy growth formulae conducted from the parameters of the transformation were as follows.

$$\text{Awajichihiro form :} \quad L_t = 56.95 \ (1 - e^{-0.244 \ (t + 0.404)})$$

$$\text{Yaminonishiki form :} \quad L_t = 61.64 \ (1 - e^{-0.216 \ (t + 0.378)})$$

Because the parameters of the Walford growth transformation between the two forms indicated significant difference from each other, at the L_t coefficient which

indicated growth rate, growth of the yaminonishiki form is far greater than that of the awajichihiro form. This might be caused by the conchological differentiation between the two forms.

Status of a pea crab ***Pinnotheres sinenis***, which was first recorded to be parasitic on the two forms of Hirase's scallop ***Volachlamys hirasei***, was examined. ***P. sinenis*** appeared in both awajichihiro and yaminonishiki forms throughout the year; in particular, the parasitism was high during winter, its expected mating period. The fact that no differences were recognized in the parasitic rate of male and female ***P. sinenis*** on the two forms in any month suggested no potential for male and female ***P. sinenis*** to select either form. Indeed, there was no potential for the whole ***P. sinenis*** to select either form. The condition factor of the parasitized ***V. hirasei*** showed similar tendencies in the two forms; namely, it was less than that of non-parasitized individuals. Further, the condition factor of the hosts parasitized by the females of ***P. sinenis***, which had much larger bodies than the males, tended to be less than that of the hosts parasitized by the males. The facts suggested that ***P. sinenis*** damaged the host ***V. hirasei*** by the parasitism, especially from the females. Those results might mediately suggest that the awajichihiro and the yaminonishiki forms are unique species.

Genetic characters of the two forms were examined using isozyme analysis. Since fitness for Hardy Weinberg equilibrium by chi square tests indicated no significance at any polymorphic loci in the awajichihiro form, the yaminonishiki form and both the forms pooled, there was no evidence that the two forms were distinct reproductive populations. Also, there were no significant differences between the two forms in the values to indicate genetic features and allelic frequencies of the 24 loci examined. The genetic distance (D value) between the two forms indicated 0.0002, being within the local population level or the identical species. The results strongly suggested that the awajichihiro and the yaminonishiki forms belong to a unique reproductive population, namely the identical species, and the morphological variabilities can be regarded as phenotypes of the genetic polymorphism. The taxonomic status was decided as follows.

Hirase's scallop (awajichihiro)	***Volachlamys hirasei*** (Bavay, 1904)
Awajichihiro form	*V.* ***hirasei*** var. ***awajiensis***
Yaminonishiki form	*V.* ***hirasei*** var. ***ecostata***

Following the study on the two forms of ***Volachlamys hirasei***, fossil specimens in genus ***Volachlamys*** from Japan were examined. The fossil specimens were from the Kazusa Formation (1.7 Ma), the Kitaarima Formation (0.9 Ma), the Maiko Formation (the Middle Pleistocene), the Takatsukayama Formation (0.41 Ma), the Atsumi Formation (0.44 Ma), the Kioroshi Formation (0.125 Ma) and a seaside allu-

vium in Takamatsu (0.006 Ma). In addition to those, living specimens of ***V. hirasei*** collected from the Seto Inland Sea were examined. Shells of the specimens were morphologically measured, and growth was analyzed by observing the annuli on the shell surfaces. In the fossil specimens, the yaminonishiki form were detected from the Atsumi, Kioroshi and Takamatsu samples; it appeared 1 of 44 individuals in the Atsumi sample, 1 of 2 individuals in the Kioroshi sample and 18 of 39 individuals in the Takamatsu sample. The remaining specimens were comprised entirely of the awajichihiro form, suggesting that the yaminonishiki form appeared around the middle Pleistocene. Morphologically, although shell proportions and costae number were unique to one another by locality, there were no samples that were particularly specialized. On the other hand, shell weight index (SWI) of the Maiko sample was prominently low, indicating that their shells were much thinner and lighter than the other specimens. The growth analysis indicated particularly greater growth of the Maiko specimens, although the other specimens (including the living species) showed similar growth rates to one another. These results suggest that only the Maiko specimens were genealogically distinct from the others. The fossil specimens of genus ***Volachlamys*** from Japan were generally supposed to be ***Volachlamys yagurai***, being treated as a distinct species from the living species of ***V. hirasei***. The specimens from the Maiko Formation, which is the locale of ***V. yagurai***, have biological characteristics so different from those of the living species that they are treated as a distinct species; however, in the other fossil specimens, significant characteristics that differ from those of the living species could not be detected. These results indicate that the fossil individuals from the Maiko Formation are ***Volachlamys yagurai***, and those from all other localities should be ***Volachlamys hirasei***, which is common to the living species.

Chapter 1 Introduction

Genus ***Volachlamys*** is a taxon of pectinid bivalves. The genus was established by Iredale (1939) with the species ***Pecten cumingii*** Reeve, which is distributed around the Indo-Pacific. In Japan, taxa of both living and fossil species exist in the genus ***Volachlamys***.

The living species in Japan consist of two forms of Hirase's scallop. One form is ***Volachlamys hirasei*** (Bavay), which is distributed only in the Seto Inland Sea (Hayami, 1985; Habe and Okutani, 1985) and the Ariake Sea (Sato, 1975; Kimura, 1977; Sato, 2000). This form was first described as ***Chlamys hirasei*** by Bavay (1904) after Yoichiro Hirase offered a specimen. The specimen, based on its description, was a so-called "yaminonishiki" (Kira, 1972; Habe, 1977); radial costae were weak and the shell surface was almost smooth. The next year, Pilsbry (1905) described a new species of ***Pecten awajiensis*** based on another specimen, also offered by Mr. Hirase. Although the latter description showed no figures of the specimen, the description itself implied that the specimen was a so-called "awajichihiro" (Kira, 1972; Habe, 1977) having a characteristic of strong costae.

Thereafter, those two "species" were taxonomically translocated into the newly established genus ***Volachlamys***. They were not regarded as a distinct species even with their morphological similarity (except for the strength of their radial costae), but instead were treated as subspecies of awajichihiro ***V. hirasei awajiensis*** (strong costa form) and yaminonishiki ***V. hirasei hirasei*** (weak costa form) (Habe and Kosuge, 1967; Kira, 1972; Habe, 1977; Habe and Okutani, 1985). Still, there were some who wanted to treat the two forms as identical species, arguing that the difference between the two forms could be regarded as an intraspecific morphological variation (Kuroda, 1932; Abbott and Dance, 1983; Matsukuma, 1986; Bernard ***et al.***, 1993; Kato and Fukuda, 1996).

Sato (1975) examined many individuals of ***V. hirasei*** from the Ariake Sea, Kyushu, and suggested that the two forms could be classified separately based on morphological characteristics of the radial costae and frequencies of color patterns. Then, Hayami (1985), who examined the specimens of the species from Osaka Bay, suggested that the differences between the two forms were merely discontinuous variations within a single population; he insisted on the need for further study to rectify this state of affairs.

Since then, there has existed some disagreement on the taxonomic treatment of the two forms, and a stable conclusion has never been given. Thus, the present study was carried out to reveal the relationship between the two forms, which were examined morphologically, ecologically and genetically.

Further, fossil bivalve specimens of the genus ***Volachlamys*** were collected from various Japanese sites and examined for phylogenetic consideration. The evolutional history of the specimens, as well as their taxonomic treatment, are discussed with the biological data of the living species.

Chapter 2 Shell morphology of awajichihiro and yaminonishiki forms

In this first study to reveal the relationship between the two forms of ***Volachlamys hirasei***, characteristics in shell morphology of the two forms were examined.

Materials and methods

Specimens examined for this study were caught via trawl in the Bisan-Seto waters of Kagawa from April, 1989 to March, 1990, and all the specimens were living (paired valves). The sampling was conducted more than once a month. The total number of collected specimens was 1187. The specimens were classified into two forms: awajichihiro (those having strong radial costae) and yaminonishiki (those with weak or no costae), using criteria of Sato (1975) and Hayami (1985). The number of awajichihiro specimens collected was 929; 258 yaminonishiki forms were found. Sampling data of the specimens are shown in Table 1. To prepare the specimens, soft parts were removed, the shells were dried, and objects adhered to their surfaces were thoroughly removed using a knife and wire brush. The left shell of each pair was measured morphologically to collect such data as shell length, shell height, shell width, auricle width, shell weight and the number of radial costae. Additionally, the width and weight of each valve pair were found. The proportions of the shell height, shell width and auricle width were expressed as percentages for the shell length. When counting the number of radial costae, any ambiguous costae were included in the count; the yaminonishiki costae were counted only when discernible. Also, in an effort to standardize the shell weight,

Table 1. Sampling data of specimens examined

Date	Awajichihiro form			Yaminonishiki form		
	N	Shell length (mm)		N	Shell length (mm)	
		Average	Range		Average	Range
Apr. 18, 1989	32	41.1	29.0 — 50.0	11	44.0	35.6 — 50.0
Apr. 19, 1989	26	43.7	32.1 — 54.0	9	46.4	37.7 — 51.5
May 2, 1989	54	44.6	34.1 — 53.6	18	45.0	35.6 — 52.8
May 12, 1989	113	42.4	30.5 — 59.2	21	46.3	33.9 — 53.1
May 25, 1989	54	46.0	36.9 — 55.0	10	46.3	39.5 — 53.1
June 2, 1989	46	46.1	35.8 — 59.2	14	48.4	39.1 — 56.2
July 2, 1989	27	47.1	37.6 — 56.4	7	44.6	39.0 — 54.5
July 3, 1989	42	46.8	37.4 — 56.6	10	45.6	36.2 — 52.2
Aug. 3, 1989	76	43.8	33.2 — 59.0	12	41.4	36.8 — 52.0
Sep. 5, 1989	93	42.1	33.8 — 57.3	21	44.0	39.0 — 52.4
Oct. 2, 1989	45	42.9	32.9 — 55.3	20	43.2	35.6 — 49.0
Nov. 7, 1989	39	41.9	29.9 — 51.2	8	42.1	37.6 — 48.7
Nov. 8, 1989	16	42.2	35.4 — 53.4	5	43.4	37.7 — 47.4
Dec. 4, 1989	37	41.0	34.5 — 52.6	25	42.6	34.0 — 50.7
Jan. 5, 1990	72	45.1	37.6 — 54.0	19	46.5	37.4 — 52.5
Feb. 4, 1990	76	46.9	38.5 — 56.9	18	48.2	42.7 — 52.5
Mar. 7, 1990	81	42.0	32.1 — 50.7	30	45.1	32.8 — 52.7
Total	929	43.8	29.0 — 59.2	258	45.0	32.8 — 56.2

shell weight index (SWI) was defined as follows.

$$SWI = SW / SL^3 \times 10^5 \quad \text{(SW: shell weight [g]; SL: shell length [mm])}$$

Further, color patterns of the shells were examined. Coloration of the left valve was classified into the eight patterns shown in Fig. 1. The details of each pattern

Fig. 1. Classification patterns of shell coloration. Words in parentheses in this explanation indicate each form shown here. 1: Pattern 1, Dappled (Awajichihiro); 2: Pattern 2, Marbled, (Awajichihiro); 3: Pattern 3, Whitish (Yaminonishiki); 4: Pattern 4, Pale brown (Yaminonishiki); 5: Pattern 5, Brown (Yaminonishiki); 6: Pattern 6, Dark purple (Yaminonishiki); 7: Pattern 7, Orange (Yaminonishiki); 8: Pattern 8, Pale brown with brown radial stripes (Yaminonishiki).

are described as follows; those individuals having intermediate coloration (between two patterns) were classified into the pattern with which they had most in common.

Pattern 1 (Dappled): Dappled with purplish dark speckles, and white or palely shaded small speckles (Fig. 1–1).

Pattern 2 (Marbled): High-contrast, white, cloud like coloration distributed in brown or dark brown base color (Fig. 1–2).

Pattern 3 (Whitish): Uniformly whitish with no coloration except the dark color on the radial costae (Fig. 1–3).

Pattern 4 (Pale brown): Pale brown or cream base color, with fine brown speckles that sometimes became radial stripes (Fig. 1–4).

Pattern 5 (Brown): Uniformly bright brown with no other remarkable coloration (Fig. 1–5).

Pattern 6 (Dark purple): Uniformly dark purple or dark purple with white or pale speckles (Fig. 1–6)

Pattern 7 (Orange): Bright orange base color with dark brown large speckles. The proportion of the orange to dark brown areas sometimes was reversed (Fig. 1–7).

Pattern 8 (Pale brown with brown radial stripes): Pale brown base color with irregularly radiating dark brown stripes, together with many fine brown speckles distributed along the growth annuli (Fig. 1–8).

Results

Table 2 summarizes the measurements of the specimens collected. Average values for the monthly specimen lots have been calculated. Also, proportions of monthly lots of the yaminonishiki form to the lots of the awajichihiro form are shown together.

The monthly average values for any characters did not vary much, and did not show any seasonal changes. Therefore, average values of the characters for all the specimens of the two forms were compared with the analysis of variance (ANOVA) and ***t***-test (Table 3). The results showed significant differences in the variance of the shell height; also, a high level of significant differences was recognized in the average values of all the characters. Thus, considerable differences in the shell morphology between the awajichihiro and yaminonishiki forms were revealed.

Frequency distribution histograms of (shell height)/(shell length), (shell width)/(shell length), SWI and radial costae are shown in Figures 2–5, respectively. As for the shell height, the awajichihiro form has a mode at about 97%, while the

Table 2. Results of morphometric measurements in awajichihiro and yaminonishiki forms

	Awajichihiro form						Yaminonishiki form						
	Shell[*1] hight	Shell[*1] width	Auricle[*1] width	SWI	Costae number	Na[*2]	Shell[*1] hight	Shell[*1] width	Auricle[*1] width	SWI	Costae number	Ny[*2]	Ny/Na
April	97.63	35.31	64.71	9.87	17.03	58	100.31	33.89	63.89	9.36	15.53	20	0.345
May	97.58	35.16	64.97	9.83	16.71	221	99.12	32.85	63.31	9.05	15.83	49	0.222
June	97.08	35.66	63.68	9.50	16.13	46	100.26	31.64	60.17	8.68	15.46	14	0.304
July	96.34	35.40	63.93	9.64	16.33	69	99.86	32.01	63.89	8.99	15.23	17	0.246
August	96.82	34.64	65.81	9.13	16.41	76	98.39	31.64	67.85	8.72	15.13	12	0.158
September	96.64	33.59	69.12	8.62	16.53	93	98.15	31.51	67.48	7.81	15.60	21	0.226
October	97.10	32.97	69.19	8.64	16.51	45	98.27	31.36	67.03	8.21	15.84	20	0.444
November	97.41	35.29	70.49	9.14	16.69	55	98.34	32.41	69.98	8.37	15.00	13	0.236
December	96.89	34.80	69.18	8.51	16.81	37	99.07	32.53	69.69	8.12	15.16	25	0.676
January	97.20	35.31	67.34	8.93	16.31	72	99.35	32.70	68.30	7.97	15.53	19	0.264
February	96.46	35.33	65.47	8.86	16.61	76	98.96	32.42	64.93	8.40	15.56	18	0.237
March	97.30	35.26	66.67	9.50	16.59	81	98.95	33.11	63.48	8.74	14.83	30	0.370
Total average	97.13	35.00	66.33	9.31	16.58	929	99.09	32.47	65.50	8.58	15.44	258	0.278

[*1] Percentage of shell length

[*2] Na: Individual numbers of awajichihiro form, Ny: Individual numbers of yanimonishiki form

Table 3. Results of ANOVA and *t* test of morphometric characters between awajichihiro and yaminonishiki forms

Form	Average value		F	*t*
	Awajichihiro	Yaminonishiki		
Shell hight*1	97.13	99.09	1.346 *	12.001 ***
Shell width*1	35.00	32.47	1.103	14.084 ***
Auricle width*1	66.33	65.50	1.058	2.173 *
SWI	9.31	8.58	1.026	8.555 ***
Costae number	16.58	15.44	1.175	11.289 ***

*1 Percentage of shell length

* Significant at 5% level *** Significant at 0.1% level

yaminonishiki form is at about 100%. The frequency distributions of the two forms differ from each other (Fig. 2). That is, the shell length tends to be larger than the shell height in the awajichihiro form, while the shell length is almost parallel with the shell height in the yaminonishiki form.

Difference in the frequency distribution between the two forms also was observed in the shell width; a discrepancy of 2–3% in the modes of the two forms was recognized (Fig. 3). In general, the shell width of the awajichihiro form is larger; specifically, swelling of both the valves of the awajichihiro form is larger than that of the yaminonishiki form. A clear difference in the frequency distribution between the two forms also was recognized in SWI. The modes of the two forms were separated by a distance of nearly 1, and the SWI values of the awajichihiro form tended to be larger (Fig. 4). Specifically, relative shell weight of the

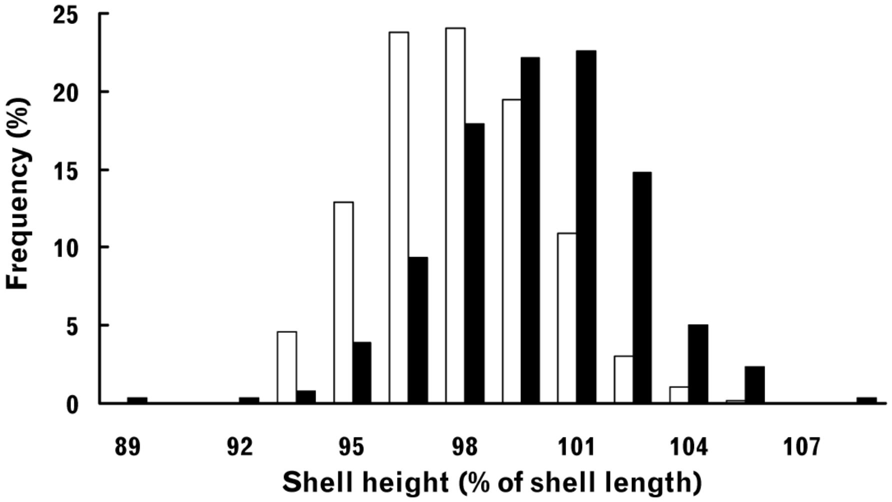

Fig. 2. Frequency distributions of SH/SL. Open bars: Awajichihiro form (929 specimens); Dark bars: Yaminonishiki form (258 specimens).

awajichihiro form was likely to be heavier than that of the yaminonishiki form. Further, the number of radial costae showed a similar tendency; the modes of the awajichihiro and yaminonishiki forms were 15 and 17, respectively. The trends of the two forms obviously differed from each other (Fig. 5).

In terms of the monthly appearance of the awajichihiro and yaminonishiki forms, frequencies of the yaminonishiki form were stable, generally being nearly 30% of the awajichihiro form (Table 2). Chi-square tests to examine the difference of the monthly frequencies showed a significance at 1% level. However, this significance occurred because the frequency of the yaminonishiki form was consider-

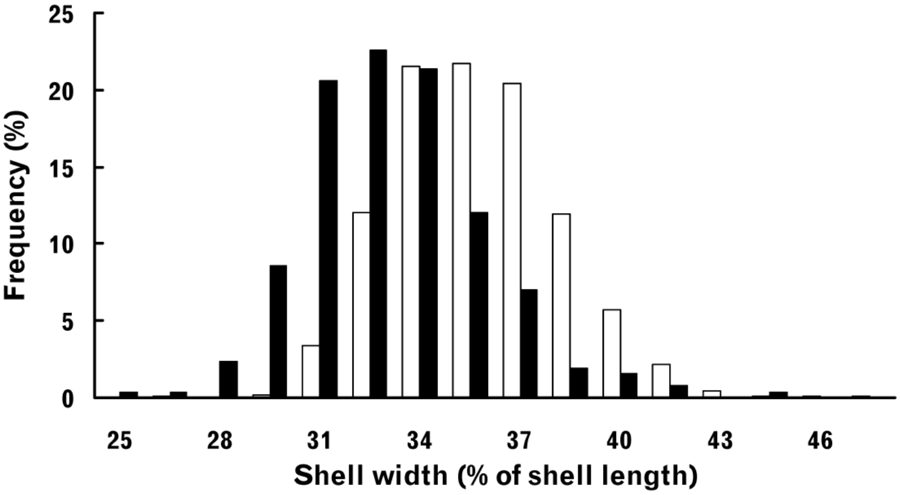

Fig. 3. Frequency distributions of SW/SL. Open bars: Awajichihiro form (929 specimens); Dark bars: Yaminonishiki form (258 specimens).

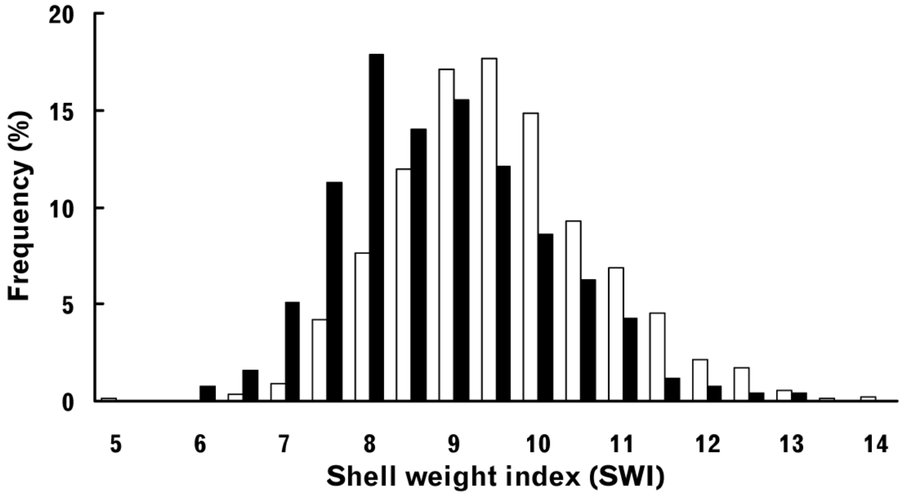

Fig. 4. Frequency distributions of SWI. Open bars: Awajichihiro form (929 specimens; Dark bars: Yaminonishiki form (258 specimens).

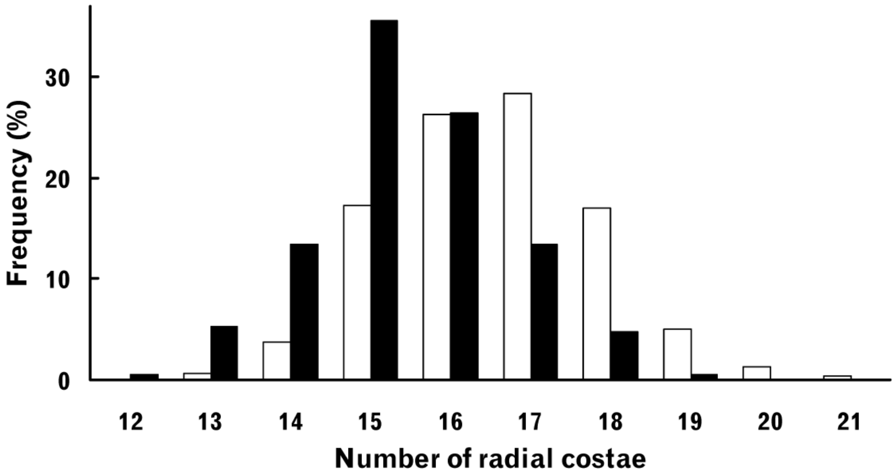

Fig. 5. Frequency distributions of radial costae. Open bars: Awajichihiro form (929 specimens); Dark bars: Yaminonishiki form (258 specimens).

ably high in the December lot (Table 2). A recalculation of the χ^2 value without the December lot indicated no significance. This suggests that the proportion of the two forms can be regarded to be universal rather than related to the seasons.

Table 4 shows monthly frequencies (percentages) of the color patterns in the awajichihiro and yaminonishiki forms. In the awajichihiro form, color pattern 1 appeared dominantly in every month; the weighted average value of color pattern 1 throughout the months indicated nearly 90%. The seven other color patterns showed low frequency; indeed, none reached 5% in average values. Among those, color pattern 2, which presented 4.4%, was the highest value. Color pattern 8 did not appear at all in the awajichihiro form. Frequency composition of the color patterns by month was similar to one another; it would be a universal tendency in the awajichihiro form (Table 4).

In the yaminonishiki form, color pattern 6 appeared dominantly in every month; the weighted average value of color pattern 6 throughout the months indicated nearly 60%. Other than color pattern 6, color patterns 1, 4, 5 and 7 indicated about 10% in frequency, while color pattern 2 did not appear at all in the yaminonishiki form. Frequency composition of the color patterns in each month was similar to one another; the universal tendency was recognized as being the same as in the awajichihiro form (Table 4).

Discussion

The results of the present study revealed that there were considerable differences in the external morphologies of the awajichihiro and yaminonishiki forms. Hayami (1985) also examined the morphological differences between the two forms,

Table 4. Percentages of color patterns in awajichihiro and yaminonishiki forms (color pattern classification refer to Fig.1)

Color	Awajichihiro form									Yaminonishiki form								
pattern	1	2	3	4	5	6	7	8	N	1	2	3	4	5	6	7	8	N
Apr.	87.9	6.9	0.0	0.0	3.4	1.7	0.0	0.0	58	25.0	0.0	0.0	10.0	0.0	45.0	15.0	5.0	20
May	86.4	5.0	1.4	3.2	2.7	0.9	0.5	0.0	221	10.2	0.0	0.0	12.2	4.1	63.3	6.1	4.1	49
Jun.	91.3	0.0	0.0	2.2	4.3	2.2	0.0	0.0	46	0.0	0.0	0.0	7.1	14.3	64.3	14.3	0.0	14
Jul.	84.1	4.3	1.4	5.8	1.4	1.4	1.4	0.0	69	0.0	0.0	0.0	17.6	17.6	58.8	5.9	0.0	17
Aug.	90.8	5.3	0.0	3.9	0.0	0.0	0.0	0.0	76	25.0	0.0	0.0	0.0	8.3	50.0	8.3	8.3	12
Sep.	86.0	5.4	0.0	5.4	1.1	2.2	0.0	0.0	93	14.3	0.0	0.0	14.3	9.5	47.6	4.8	9.5	21
Oct.	97.8	0.0	0.0	0.0	0.0	0.0	2.2	0.0	45	0.0	0.0	5.0	5.0	10.0	70.0	10.0	0.0	20
Nov.	83.6	5.5	0.0	9.1	0.0	0.0	1.8	0.0	55	7.7	0.0	0.0	15.4	0.0	53.8	15.4	7.7	13
Dec.	83.8	10.8	0.0	5.4	0.0	0.0	0.0	0.0	37	4.0	0.0	0.0	24.0	16.0	44.0	12.0	0.0	25
Jan.	90.3	2.8	0.0	2.8	0.0	2.8	1.4	0.0	72	0.0	0.0	5.3	21.1	15.8	52.6	5.3	0.0	19
Feb.	90.8	2.6	1.3	2.6	2.6	0.0	0.0	0.0	76	5.6	0.0	0.0	0.0	5.6	77.8	11.1	0.0	18
Mar.	86.4	3.7	0.0	2.5	1.2	6.2	0.0	0.0	81	6.7	0.0	0.0	0.0	0.0	70.0	23.3	0.0	30
total	87.8	4.4	0.5	3.6	1.6	1.5	0.5	0.0	929	8.1	0.0	0.8	10.9	7.8	58.9	10.9	2.7	258

and pointed out that the awajichihiro form had larger shell length than shell height, and had better swelled and relatively heavier shells than those of the yaminonishiki form. These characteristics correspond well with the results in the present study.

Regarding the point that the awajichihiro form had better swelled and relatively heavier shells than the yaminonishiki form, Hayami commented that the differentiation in these two characters might indirectly express the difference of costae strength between the two forms. His opinion is quite supportable; the strong corrugated costae of the awajichihiro form can make shell surface area broader, subsequently making shell volume larger and making shell weight heavier than those of the yaminonishiki form.

However, Hayami mentioned that there was no significant difference in the number of radial costae between the two forms, and this point does not correspond with the results in this chapter. Thus, a trial was performed to read frequency distribution from his histogram of the costae number, and the average values of the costae number for the two forms were calculated. The average values resulted in 16.13 and 15.50 for the awajichihiro and yaminonishiki forms, respectively, corresponding well with the results in the present study (Table 2). Further, ***t***-test for the average values of the two forms resulted in t=2.171 (P=0.033), being significant at the 5% level. This indicates that the difference in the costae number existed also in a small number of the specimens examined by Hayami.

The reason the yaminonishiki form has fewer radial costae appears to be that the costae located in the anterior and posterior margins of the shell are particularly ambiguous as a result of the costae weakness. This phenomenon can express the difference of costae strength by another characteristic rather than by one of the morphological differences as mentioned by Hayami (1985).

As for the frequency of the awajichihiro and yaminonishiki forms, Sato (1975), who performed continuous sampling in the Ariake Sea, mentioned that he found the general ratio of awajichihiro to yaminonishiki forms to be 3 to 1, at first. However, in a later sampling, Sato commented that the yaminonishiki were not always less, reporting 174 awajichihiro and 144 yaminonishiki forms from the sampling taken on May 25, 1975.

Although the initial ratio of 3 to 1 corresponds well with the results in the present study, the results on May 25, 1975 fairly differs. One could hypothesize that ***Volachlamys hirasei*** in the Ariake Sea might be a distinct population from the one in the Seto Inland Sea; otherwise, the frequency composition of the two forms in the sampled specimens on May 25, 1975 might differ by chance from that of the norm, as was the case in the December, 1989 collection for the present study (Table

2). Hayami (1985) examined 112 individuals of ***V. hirasei*** from Osaka Bay, reporting 59 yaminonishiki and 53 awajichihiro forms. He regarded the rates of the two forms to be nearly halves, inferring a potential that the frequencies of the two forms differ by locality.

The color pattern composition in each form was universal and both compositions were considerably different from each other (Table 4). Further, there were unique color patterns, which did not appear in the other form, such as color pattern 8 in the awajichihiro form and color pattern 2 in the yaminonishiki form (Table 4).

According to the results from the specimens of the Ariake Sea examined by Sato (1975), coloration of the awajichihiro form was mostly dark brown (consistent with color pattern 1 in the present study), with slight variation in shading, but without major differences in coloration; the yaminonishiki form was mainly purplish (consistent with color pattern 6 in the present study), with much variation in shading and including orange coloration (consistent with color pattern 7 in the present study). This report corresponds well with the results in the present study (Table 4). Sato also suggested a negative opinion of Kira (1972), who described the coloration of the two forms as being totally the same. The results of the present study also absolutely refute Kira's descriptions (1972); when regarding the two forms as populations, it is obvious that the coloration of the two forms differ from each other. Also, present findings cast doubt on the statement made by Hayami (1985) that a difference in the coloration of the two forms was not observed.

Thus, it was clarified that there are considerable differences in the external morphologies of the awajichihiro and yaminonishiki forms. Also in the present study, the two forms could be completely identified with the strength of the radial costae as pointed out by Sato (1975) and Hayami (1985). In terms of morphological taxonomy, these criteria might support treating the two forms as subspecies (Habe and Kosuge, 1967; Kira, 1972; Habe, 1977; Habe and Okutani, 1985). However, it would be premature to draw a conclusion based only on the morphological results discussed in this chapter. Therefore, further studies on other biological characteristics were performed and will be discussed in the chapters that follow.

Chapter 3 Soft part morphology and seasonal changes of awajichihiro and yaminonishiki forms

To reveal the relationship between the two forms (awajichihiro and yaminonishiki) of ***Volachlamys hirasei***, Chapter 2 examined the shell morphology characteristics of the two forms, showing considerable differences between them. Pertaining to the subsequent study, this chapter reports on soft part morphology and seasonal changes of the two forms.

Materials and methods

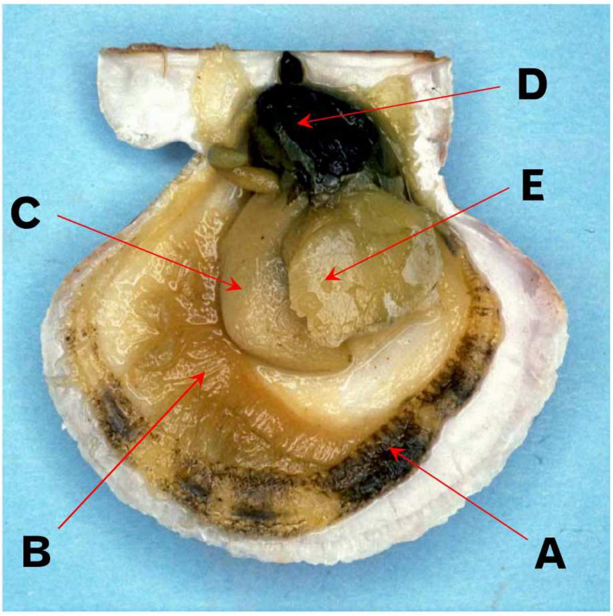

Fig. 6. Internal characters of ***Volachlamys hirasei***. **A**: Mantle; **B**: Gill; **C**: Gonad; **D**: Digestive diverticulum; **E**: Adductor muscle.

Specimens for study in this chapter were the same ones examined in Chapter 2; they were caught via trawl in the Bisan-Seto waters of Kagawa from April, 1989 to March, 1990. The specimens were classified into the awajichihiro and yaminonishiki forms with the criteria described in Chapter 2. The specimens first were stocked in a refrigerator; thereafter, they were fixed with 10% formalin, then placed into 80% ethanol and preserved.

Figure 6 shows the general morphology of the soft part of ***Volachlamys hirasei***. It is quite similar to that of such other pectinid shells as the Japanese baking scallop, ***Pecten albicans*** (Shiino, 1969), the blistered scallop, ***Cryptopecten vesiculosus*** (Hayami, 1984), the yesso scallop, ***Patinopecten yessoensis*** (Mori, 1989) and the Pacific pink scallop, ***Chlamys hastata hericia*** (Habe, 1994).

To measure each specimen, the soft part was removed from the shells and the wet weight of the soft part was taken. To standardize the soft part weight, condition factor (CF) was calculated with the following formula.

$$CF = BW / SL^3 \times 10^5 \quad \text{(BW: soft part weight [g]; SL: shell length [mm])}$$

Subsequently, pairs of mantles and gills, the gonad, the digestive diverticulum and the adductor muscle were removed from the soft part; the wet weight was obtained for each part. For standardization, an index of each part was given, including its percentage of the total soft part weight. Further, adductor muscle traces inside the shells were examined; larger and shorter diameters of the traces in both shells were measured, and percentages of the shell length were calculated. Although the gonads were anatomized and observed with a microscope, individual sexes could not be identified precisely; therefore, the sexes were not distinguished.

Results

Soft part morphology

As another basis of comparison of the soft parts of the awajichihiro and yaminonishiki forms, all the specimens were carefully observed visually throughout the sampling period. However, the morphology of the soft parts of the two forms was found to be quite similar as shown in Figure 6. Any morphological differentiation by form was not recognized at all.

Condition factor

Figure 7 shows seasonal changes of condition factor (CF) of the two forms. Monthly average values of the two forms were similar to each other, indicating quite similar changing patterns. CF hardly changed from April to June, with a value of about 5. After July, CF gradually decreased until September, when it showed its minimum value. Thereafter, CF gradually increased until January/

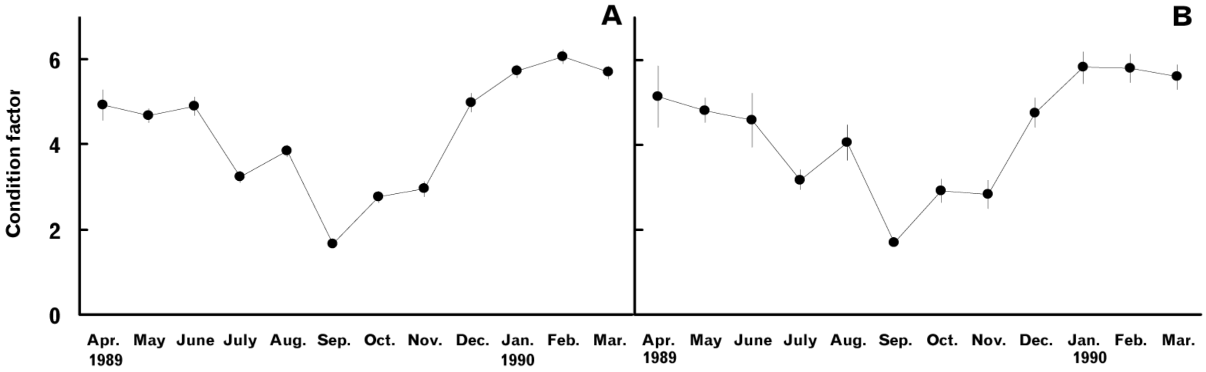

Fig. 7. Seasonal changes of condition factor (CF). A: Awajichihiro form; B: Yaminonishiki form. Dark circles indicate average values of all the specimens examined in each month, longitudinal bars indicate 95% confidence intervals of each average value.

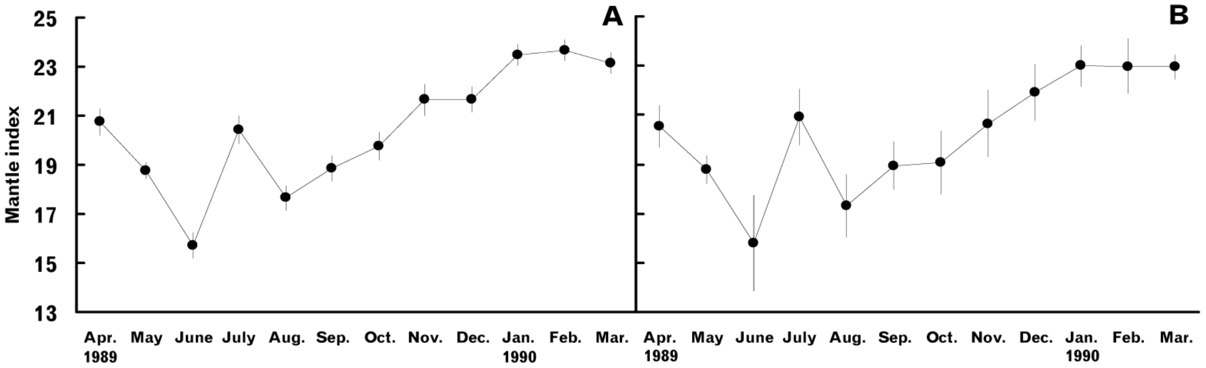

Fig. 8. Seasonal changes of mantle index (MI). A: Awajichihiro form; B: Yaminonishiki form. Dark circles indicate average values of all the specimens examined in each month, longitudinal bars indicate 95% confidence intervals of each average value.

February. Thus, throughout the year, CF is high from winter to spring and low from summer to autumn.

Mantle

Figure 8 shows seasonal changes of mantle index (MI) of the two forms. Monthly average values of the two forms were very close to each other, indicating almost equivalent fluctuation patterns. MI decreased from April to June, showed a sudden increase in July (back to April's level), and decreased again in August. Thereafter, MI steadily and uneventfully increased to January, subsequently showing almost no fluctuation until March. Assuming that the values in July were abnormal, it seems that MI reduces in summer and increases from autumn to winter.

Gill

Figure 9 shows seasonal changes of gill index (GI) of the two forms. GI also indicated quite similar fluctuation patterns in the two forms. GI decreased from April to June; it then switched and began increasing, showing maximum values in

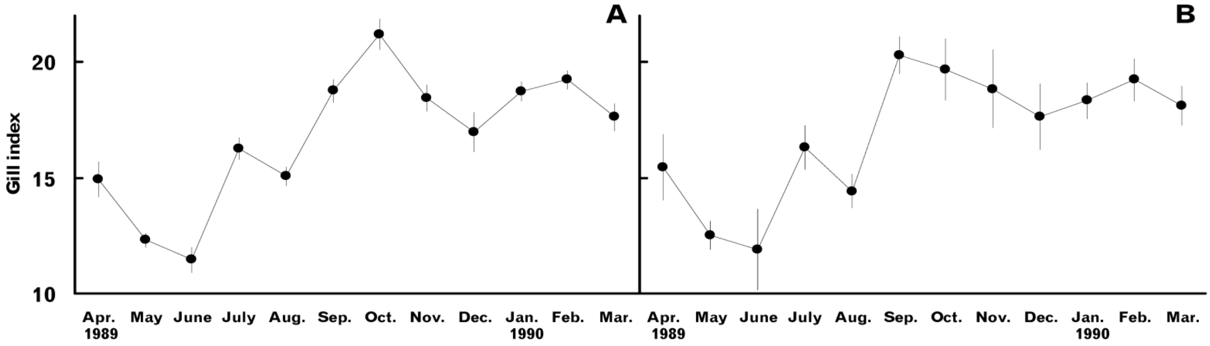

Fig. 9. Seasonal changes of gill index (GI). A: Awajichihiro form; B: Yaminonishiki form. Dark circles indicate average values of all the specimens examined in each month, longitudinal bars indicate 95% confidence intervals of each average value.

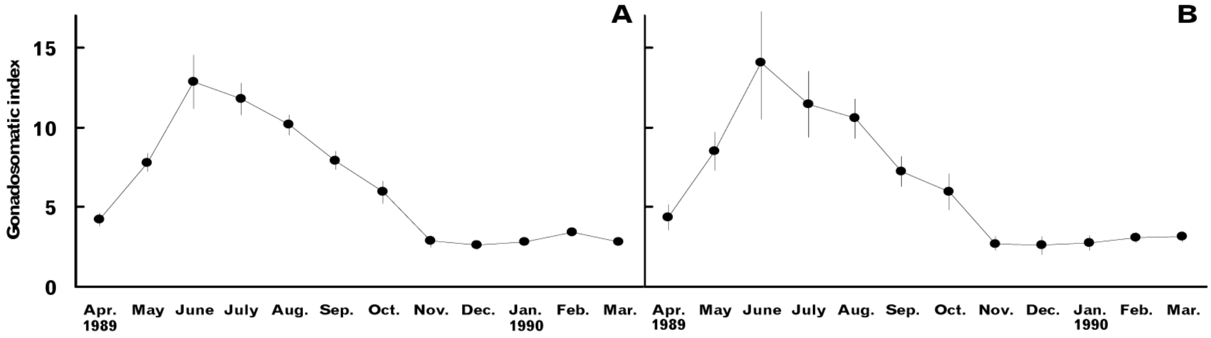

Fig. 10. Seasonal changes of gonadosomatic index (GSI). A: Awajichihiro form; B: Yaminonishiki form. Dark circles indicate average values of all the specimens examined in each month, longitudinal bars indicate 95% confidence intervals of each average value.

September/October. Thereafter, GI tended to decrease until December, showed a slight increase to February, and slightly decreased again in March.

Gonad

The relative size of the gonad generally is called gonado-somatic index (GSI); its fluctuation is closely related to the animal's reproductive cycle (Mori, 1989; Uki, 1989). Figure 10 shows seasonal changes of GSI of the two forms. GSI indicated very similar fluctuation patterns in the two forms. GSI rose up from April and indicated a peak in June, with an average value of nearly 15. Thereafter, GSI gradually decrease until November, and was nearly stable in low values until March. The fluctuation of GSI implies that both forms are involved in reproductive activity from May to October.

Digestive diverticulum

In bivalve shells that feed mainly on plankton, relative size of the digestive diverticulum is greatly influenced by feeding amount. Therefore, the present

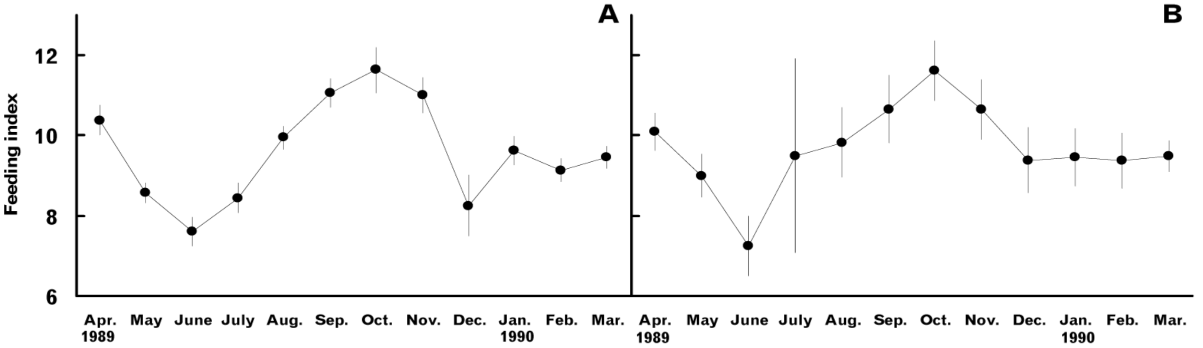

Fig. 11. Seasonal changes of feeing index (FI). A: Awajichihiro form; B: Yaminonishiki form. Dark circles indicate average values of all the specimens examined in each month, longitudinal bars indicate 95% confidence intervals of each average value.

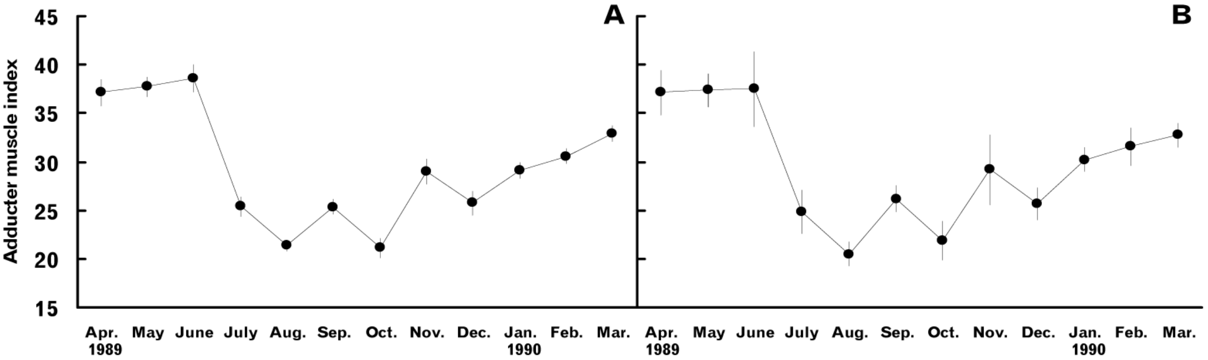

Fig. 12. Seasonal changes of adductor muscle index (AMI). A: Awajichihiro form; B: Yaminonishiki form. Dark circles indicate average values of all the specimens examined in each month, longitudinal bars indicate 95% confidence intervals of each average value.

study defines the weight percentage of the digestive diverticulum of the soft part as feeding index (FI). Figure 11 shows seasonal changes of FI of the two forms. Although the confidence intervals of the yaminonishiki form were largely owing to fewer numbers of individuals, FI also indicated similar fluctuation patterns in the two forms. FI decreased from April to June and showed the minimum values in June. Thereafter, it began to increase steadily and uneventfully, and showed maximum values in October. Thereafter, FI decreased again until December, and remained almost stable until March. The fluctuation of FI infers that both forms feed very little in June especially, and are inclined to feed most in autumn.

Adductor muscle

Figure 12 shows seasonal changes of adductor muscle index (AMI) of the two forms. AMI also indicated almost equivalent fluctuation patterns in the two forms, as in the cases of the other items. AMI hardly changed from April to June in the high-value level, subsequently dropped rapidly until August, when the values were nearly one-half of those in April through June. Thereafter, AMI tended to fluctuate slightly, gradually increasing until March. On an annual basis, AMI appeared to be high from spring to early summer and low from summer to autumn.

Adductor muscle trace

Table 5 summarizes monthly results of the measurement of the adductor muscle traces of the two forms. In both the forms, the longer and shorter diameters of the adductor muscle traces tended to be larger from spring to early summer and smaller from summer to autumn, corresponding well with the fluctuation pattern of adductor muscle index (AMI) (Fig. 7). The values of (shorter diameter)/(longer diameter) (S/L) did not infer any seasonality in both valves of the two forms (Table 5).

When comparing the left and right valves, a universal phenomenon was discovered: both the longer and shorter diameters of the adductor muscle trace were larger in the left valves than in the right valves (Table 5). In contrast, the S/L values of the right valves were inclined to be larger than the S/L values of the left valves (Table 5). Such attributes as seasonal changes of the longer and shorter diameters, and relative sizes of the diameters in the left and right valves, were very similar between the two forms.

Discussion

Condition factor (CF) of the two forms of ***Volachlamys hirasei*** indicated seasonal changes; it was high from winter to spring and low from summer to autumn (Fig. 7). This relates closely to the reproductive cycle because the fluctuation of CF clearly shows a negative correlation with that of gonado-somatic index (GSI) (Fig.

Table 5. Results of measurements of adductor muscle trace inside the shell of awajichihiro and yaminonishiki forms

	Awajichihiro form						Yaminonishiki form					
	Left valve			Right valve			Left valve			Right valve		
	L[*1]	S[*2]	S/L[*3]	L[*1]	S[*2]	S/L[*3]	L[*1]	S[*2]	S/L[*3]	L[*1]	S[*2]	S/L[*3]
April	39.09	24.48	62.61	32.42	22.95	70.86	38.50	24.15	62.72	32.85	23.09	70.40
May	39.22	24.98	63.75	32.82	23.93	73.13	38.94	25.15	64.67	33.51	24.52	73.42
June	38.88	24.45	62.99	33.01	22.89	69.49	37.99	25.65	67.57	33.81	23.51	69.63
July	37.20	24.07	64.69	30.82	23.06	74.81	37.78	24.52	64.89	32.21	24.11	74.86
August	35.83	20.81	58.13	30.20	18.71	62.22	33.91	20.09	59.54	28.43	18.50	65.40
September	34.45	21.52	62.54	27.47	20.84	76.00	34.39	22.09	64.29	27.55	20.70	75.37
October	32.73	19.88	60.84	27.95	17.57	62.94	33.52	20.12	60.05	28.27	17.61	62.36
November	35.38	22.54	63.78	28.92	20.79	71.93	35.02	22.81	65.28	29.55	21.44	72.52
December	34.62	22.37	64.93	30.59	19.25	63.32	34.60	22.10	64.10	30.62	20.28	66.34
January	36.35	22.01	60.72	31.85	19.28	60.60	36.25	22.59	62.38	32.63	19.54	59.96
February	37.05	23.07	62.37	31.26	21.12	67.64	36.88	23.75	64.49	31.53	21.45	68.17
March	38.15	24.63	64.65	32.54	23.42	72.06	37.82	25.07	66.42	32.73	23.54	71.92
Total average	37.05	23.25	62.79	31.10	21.67	69.81	36.67	23.49	64.08	31.50	21.95	69.76

[*1] Percentage of adductor muscle trace longer diameter for shell length

[*2] Percentage of adductor muscle trace shorter diameter for shell length

[*3] Percentage of shorter diameter for longer diameter

Table 6. Results of ANOVA and *t* test of adductor muscle trace measurements between awajichihiro and yaminonishiki forms

Form		Average value		F	*t*
		Awajichihiro	Yaminonishiki		
Left valve	L[*1]	37.05	36.67	1.012	1.785
	S[*2]	23.25	23.49	1.002	1.358
	S/L[*3]	62.79	64.08	1.09	3.767 ***
Right valve	L[*1]	31.10	31.50	1.031	2.004
	S[*2]	21.67	21.95	1.039	1.406
	S/L[*3]	69.81	69.76	1.125	0.105

[*1] Percentage of adductor muscle trace longer diameter for shell length

[*2] Percentage of adductor muscle trace shorter diameter for shell length

[*3] Percentage of shorter diameter for longer diameter

*** Significant at 0.1% level

10).

The gonad requires much nutrition during the spawning season; subsequently, nutrition for other body parts is reduced, and consequently the weight of the soft part goes down. In particular, the fact that feeding index (FI) indicates the minimum values in June (Fig. 11) when GSI indicates a peak (Fig. 10) can be one of the factors that causes the shortage of nutrition. The reason the feeding amount drops to near inactivity during the spawning season is because much energy and time is required for reproductive activity. Subsequently, when the spawning season has passed, FI gradually increases relative to the decrease of GSI (Fig. 11), and the CF value returns to the level before the spawning season (Fig. 7) as a consequence.

According to the reports on the gonad maturation and condition factor of the yesso scallop, ***Patinopecten yessoensis***, which also belongs to the family Pectinidae, seasonal changes of the gonad maturation and that of the condition factor correspond well to each other (Maru and Obara, 1973; Sato ***et al.***, 1993), and are not consistent with the case of the two forms of ***V. hirasei*** examined in the present study. The reason for this is thought to be that the gonad weight of ***P. yessoensis*** increases at most to about 30% of the soft part; subsequently, the soft part weight is greatly influenced by the gonad weight (Maru and Obara, 1973), meaning the soft part weight (except the gonad) reduces during the spawning season by consumption of energy for the spawning (Maru and Obara, 1973). This is confirmed by the fact that the amount of glycogen in the adductor muscle, which is a source of the energy, is less in the spawning season, and rapidly increases after the spawning season (Ichisugi ***et al.***, 1972).

In the case of the two forms of ***V.*** ***hirasei*** in the present study, the gonad weight was at most about 15% of the soft part, being about one-half of the case of ***P.*** ***yessoensis*** (Fig. 10). Therefore, in ***V.*** ***hirasei,*** there appears to be less influence of the gonad weight on the soft part weight; considerable diminishment of the soft part (including the adductor muscle) would cause the significant reduction of CF in the spawning season as shown in Figure 7. To calculate the CF values, if they are calculated with a denominator from which the gonad weight is deducted, the seasonal fluctuation of CF shown in Figure 7 would be close to clearer cyclic function curve.

The fact that the condition factor reduces because of the development of the gonad also is reported in some fishes such as the marbled rockfish, ***Sebastiscus marmoratus*** (Yokogawa ***et al.***, 1992a), the black rockfish, ***Sebastes inermis*** (Yokogawa ***et al.***, 1992b) and the Spanish mackerel, ***Scomberomorus niphonius*** (Yokogawa, 1996), making it probable that this is a common phenomenon to many organisms.

Concerning the correlation between the gonad maturation and condition factor, adductor muscle index (AMI) indicated rapid decrease in June/July (Fig. 12), suggesting the significant diminishment of the adductor muscle in the soft part. The diminishment of the adductor muscle also is well indicated in the changes of the adductor muscle trace (Table 5). Before the diminishment, mantle index (MI) and gill index (GI) indicated the minimum values in June; subsequently, they returned gradually to the levels before the spawning season (Figs. 8, 9). The seasonal changes of MI and GI could be influenced also by the changes of the gonad (Fig. 10).

The seasonal changes of GSI of the two forms implies that both of them perform reproductive activity from May to October (Fig. 5). According to reports that examined the spawning seasons of the pectinid shells, it is from April to July in the yesso scallop, ***Patinopecten yessoensis*** (Chang ***et al.***, 1985), from June to mid July in the giant Pacific scallop, ***Patinopecten caurinus*** (Chang ***et al.***, 1985), from August to October in the Atlantic deep sea scallop, ***Placopecten magellanicus*** (Chang ***et al.***, 1985), from June to early July in the Iceland scallop, ***Chlamys islandica*** (Chang ***et al.***, 1985), from April to July in the akazara scallop, ***Chlamys nipponensis akazara*** (Tanita, 1979), from late January to early May in the Japanese baking scallop, ***Pecten albicans*** (Satake and Moriwaki, 1981), and from July to November in the blistered scallop, ***Cryptopecten vesiculosus*** (Takenaka, 1999), while in the noble scallop, ***Chlamys nobilis,*** there are two spawning seasons during the year, from June through July and from October through December (Komaru and Wada, 1988).

In summary, the spawning season of the many pectinid shells is from spring through summer, when the water temperature is rising. With respect to the two forms of ***V.*** ***hirasei*** examined in the present study, their data corresponds well with the universal tendency of the family Pectinidae. In particular, it is notable that the spawning period of time is very long in the two forms of ***V.*** ***hirasei*** (Fig. 10), inferring the possibility that single individuals perform multiple spawnings within a single spawning season.

Thus, every part in the soft part of the two forms of ***V.*** ***hirasei*** indicates formulated seasonal change patterns, which can be influenced greatly by the reproductive cycle. Further, the seasonal change patterns of the two forms are very similar to each other (Figs. 7–12, Table 5). In addition, no difference was detected in the soft part morphology.

These results imply a hypothesis that the awajichihiro and yaminonishiki forms are the same reproductive populations. The facts that the two forms are sympatric (Sato, 1975; Hayami, 1975) and have identical reproductive seasons (Fig. 10) strongly support the hypothesis. This quite contradicts the morphological results in Chapter 2, in which considerable differences in the external morphology were detected between the two forms.

Even considering the results of Chapters 2 and 3, biological information was deemed insufficient to make a final determination on the taxonomic treatment of the awajichihiro and yaminonishiki forms. Therefore, further studies from other viewpoints were performed.

Chapter 4 Age and growth of awajichihiro and yaminonishiki forms

To reveal the relationship between the two forms (awajichihiro and yaminonishiki) of ***Volachlamys hirasei***, Chapter 2 examined the differences in the external morphology of the two forms, indicating considerable morphological differences between the two forms. Further, Chapter 3 examined the internal characters of the two forms and their seasonal changes, reporting no difference in the internal morphology between the two forms and considerable similarity in their seasonal change patterns. Subsequently, this chapter reports on age and growth of the two forms.

Materials and methods

Specimens for this chapter were the same ones examined in Chapters 2 and 3, being caught via trawl in the Bisan-Seto waters of Kagawa from April, 1989 to March, 1990. The specimens were classified into the awajichihiro and yaminoni-

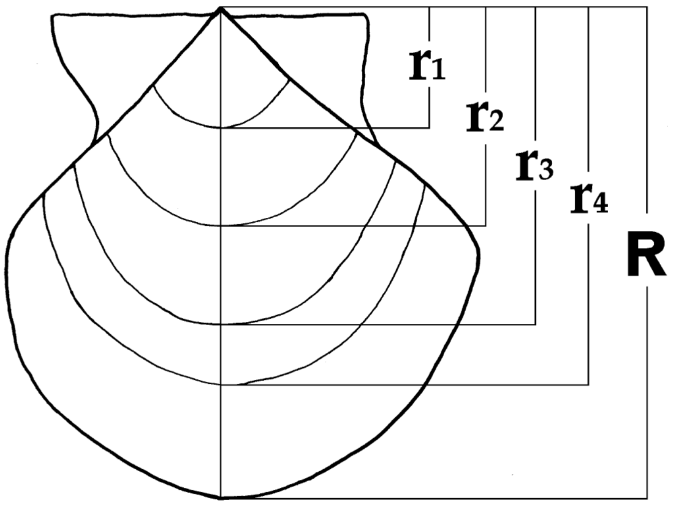

Fig. 13. Measuring method of annuli.

shiki forms with the criterion discussed in Chapter 2. To prepare the specimens, soft parts were removed, the shells were dried and objects adhered to their surfaces were removed thoroughly with a knife and wire brush.

Annuli observed on the shell surfaces were used as an age indicator. Diameters of the annuli on the left valves were measured with calipers as shown in Figure 13; namely, a vertical line was drawn from the umbo to the lower margin of the shell; the distance to each intersection point with each annulus was defined as annulus diameter (r_1, r_2, r_3, ････), and the distance from the umbo to the lower margin of the shell was defined as R, which corresponds with the shell height.

For calculation of marginal growth rate (MGR), usual methods used the formula as follows.

$$MGR = (R - r_n) / (r_n - r_{n-1})$$

This expresses the amount of growth after the last annulus (marginal growth amount) as the rate of growth amount between the two annuli just before the last annulus (usually yearly growth amount). However, in this case, the denominator values rather vary by individual, particularly in aged individuals that grow sluggishly; a slight difference in the denominator values, and an error in the measurement can affect the MGR values greatly.

Therefore, the present study expressed the amount of growth after the last annulus (marginal growth amount) as the rate of standard growth amount expected until the next annulus is formed, with the formula as follows.

$$MGR = (R - r_n) / (r_{n+1} - r_n)$$

Where, r_{n+1} is the expected value given with the substitution of the last r_n value for the correlated formula of r_n with r_{n+1}, which is calculated with the least square method.

For the calculated individual MGR of the two forms, monthly frequency distribution in 0.1 intervals were produced, and 1 or 2 normal distributions were applied by using a BASIC program after Akamine (1985); subsequently, modes (average values) of the normal distributions were computed. Time of annulus formation was expected with the seasonal changes of the MGR modes.

Results and discussion

Seasonal changes of frequency distributions of shell length

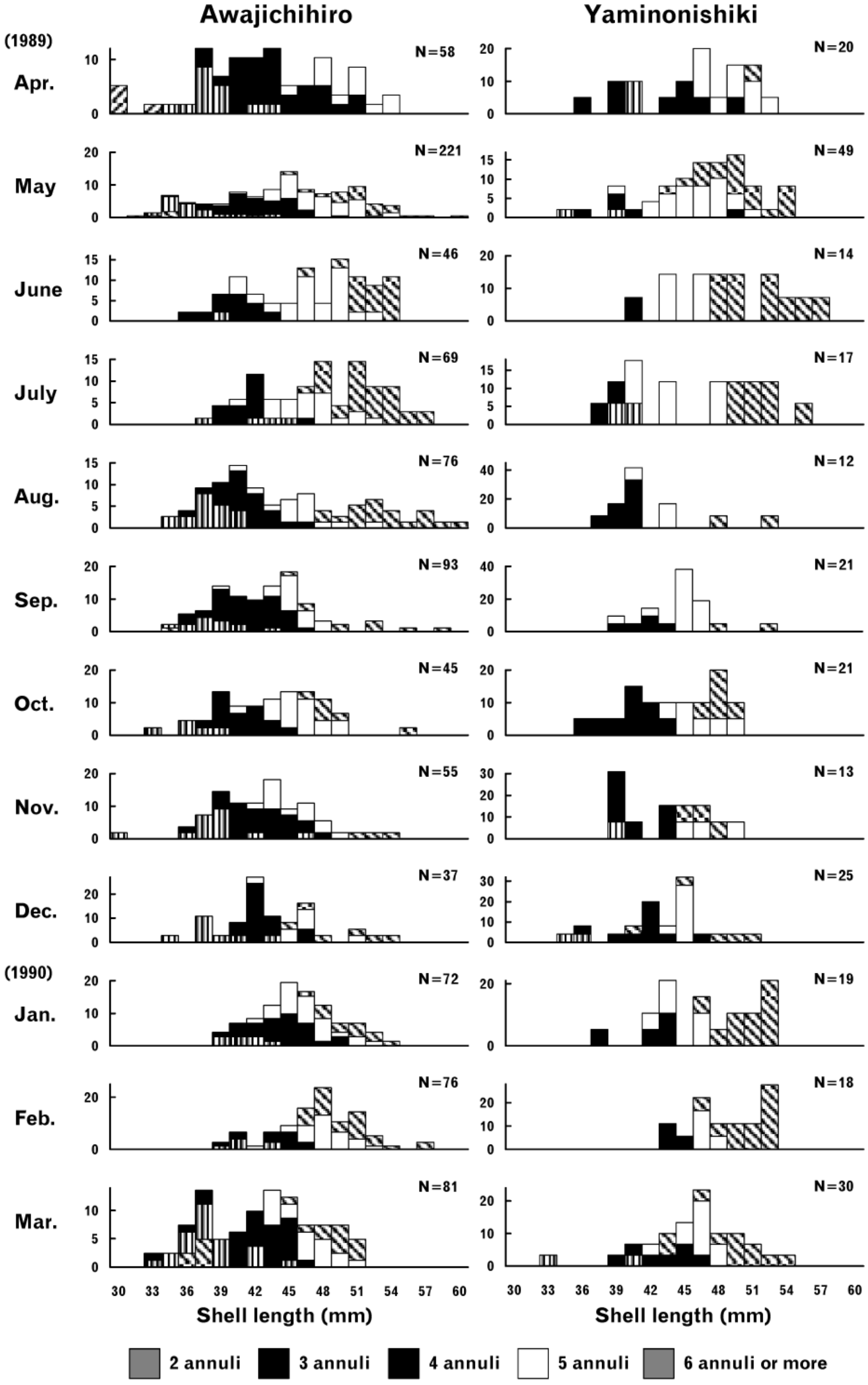

Fig. 14. Seasonal changes of shell length compositions. Longitudinal axes indicate frequencies (%) of individuals.

For the awajichihiro and yaminonishiki forms, Figure 14 illustrates monthly histograms of frequency distributions of shell length in 1.5 mm intervals with compositions of annulus groups. The histograms of both the forms indicated polymodal distributions in every month; many of the modal distributions consisted of almost single annulus groups. Although the frequency distribution of the each annulus group has a certain range, the frequency distribution of single annulus groups are well convergent and hardly indicates the distribution far from such convergence. Further, mode of each annulus group tends to increase steadily from June to next March, suggesting that each group is growing. These facts imply that the annulus groups stand for the year class groups.

Time of annulus formation

For the calculation of marginal growth rate (MGR), the correlated formulae of r_n with r_{n+1} of the awajichihiro and yaminonishiki forms were calculated with the least square method by using all data of the individuals examined. Both the forms showed high correlation between r_n and r_{n+1}, given the formulae as follows.

Awajichihiro form $r_{n+1} = 0.783\, r_n + 12.93$ ($r = 0.974$, $N = 3291$)

Yaminonishiki form $r_{n+1} = 0.806\, r_n + 12.18$ ($r = 0.982$, $N = 1066$)

By using the above formulae, seasonal changes of MGR were examined with the methods as earlier mentioned (Fig. 15). MGR of the two forms indicated similar seasonal change patterns to each other, indicated two modes from April to June, which were low values less than 0.4 and high values more than 0.8. Thereafter, MGR was almost stable in a level of about 0.5 from July to October, and subsequently increased to about 0.8 from November to March. In March, the second mode appeared again in about 0.4.

The fluctuation of MGR can be interpreted as follows.

From March to June, there are two MGR groups, one of which has high MGR values, indicating it is just before the annulus formation; another group has low MGR values, indicating it is just after the annulus formation. The stableness of

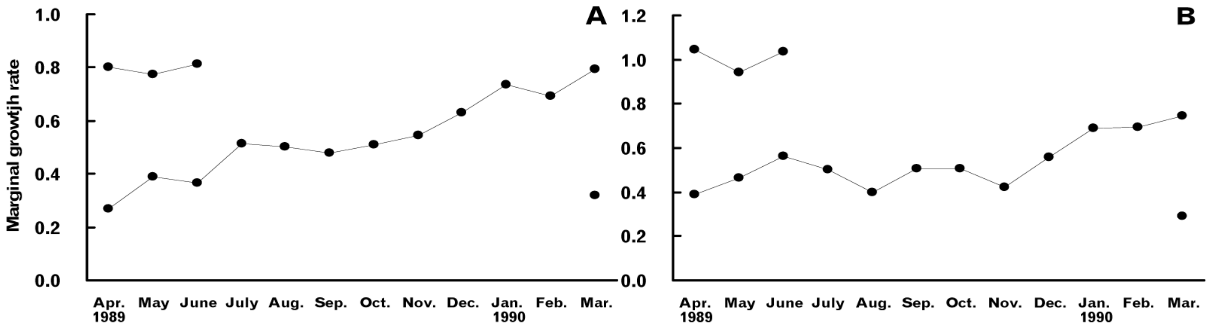

Fig. 15. Seasonal changes of marginal growth rates (MGR).
A: Awajichihiro form, B: Yaminonishiki form.

MGR from July to October implies stagnancy of the growth. Thereafter, MGR increased from November to March, indicating that the shells were growing during the period. In March, a new group with low MGR value appeared, suggesting that new annuli had formed.

Thus, the annuli observed on the shell surfaces can be regarded as "winter rings," which are formed once a year from spring to early summer. The transition of frequency distributions of shell length (Fig. 14) also infers that the annuli indicate the age. Using the annuli in the two forms of ***V. hirasei*** as the age indicator is suggested.

Although there are not many reports on the growth of shells as analyzed with the age indicator, using the annuli in the shells as the age indicator was reported in some bivalve shells such as the common blue mussel, ***Mytilus galloprovincialis*** (Hosomi, 1989), the hard-shelled mussel, ***Mytilus coruscus*** (Ito ***et al.***, 1997), the yesso scallop, ***Patinopecten yessoensis*** (Maru and Obara, 1967), the blistered scallop, ***Cryptopecten vesiculosus*** (Takenaka, 1999), the Japanese corbicula, ***Corbicula japonica*** (Utoh, 1981), the Japanese cockle, ***Fulvia mutica*** (Tateishi ***et al.***, 1977; Uchino and Tsuji, 1991), the Japanese common clam, ***Ruditapes philippinarum*** (Ito ***et al.***, 1987), the Japanese mactra, ***Mactra sulcataria*** (Hanaoka and Shimadzu, 1949) and the Japanese surf clam, ***Spisula sachalinensis*** (Sasaki, 1993). Therefore, the annuli in the bivalve shells appear to be a useful age indicator, like scales, otoliths and vertebrae in fishes (Kubo and Yoshihara, 1957; Ochiai, 1979a).

Estimation of growth formulae

To convert the measured annulus diameters into the shell length at the annulus formation, correlation formulae of shell height (SH) with shell length (SL) initially were calculated with the least square method, and the following formulae were given.

Awajichihiro form $SL = 1.061\ SH - 1.312$ $(r = 0.982,\ N = 929)$

Yaminonishiki form $SL = 0.978\ SH + 1.420$ $(r = 0.968,\ N = 258)$

For the awajichihiro and yaminonishiki forms, the shell length at each annulus formation was calculated with these formulae, and summarized by annulus group (Tables 7, 8). The shell length at annulus formation tended to be smaller in aged individuals, and was particularly obvious in the awajichihiro form (Table 7).

This can be regarded as a kind of Lee phenomenon, in which the size of younger individuals estimated from annulus diameters of aged individuals is relatively smaller (Ochiai, 1979b). The causes expected by Ochiai (1979b) are that larger individuals may be selectively collected in younger age classes, or within a single age class, while smaller individuals may overcome fishing pressure and have lower natural mortality, consequently showing a higher survival than the larger ones.

Table 7. Average values of estimated shell lengths in each annulus group in awajichihiro form

Annulus group	N	Estimated shell length (mm) ± Standard deviation (unbiased value)							
		r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8
1	0								
2	17	17.23 ± 2.00	27.56 ± 1.50						
3	138	16.75 ± 2.26	25.85 ± 2.42	33.00 ± 1.99					
4	317	16.58 ± 2.28	25.13 ± 2.54	32.00 ± 2.01	37.40 ± 2.05				
5	276	16.45 ± 2.18	24.99 ± 2.34	32.06 ± 2.19	37.59 ± 2.15	41.56 ± 2.18			
6	141	16.39 ± 2.22	24.64 ± 2.24	31.89 ± 1.88	37.49 ± 1.92	41.72 ± 1.94	44.90 ± 2.06		
7	36	16.08 ± 2.03	24.20 ± 2.13	31.33 ± 2.15	37.10 ± 2.18	41.67 ± 1.93	45.42 ± 2.01	48.03 ± 2.16	
8	4	17.06 ± 1.66	25.56 ± 2.67	31.35 ± 1.12	36.78 ± 0.88	41.08 ± 1.09	45.26 ± 0.85	48.60 ± 2.18	51.46 ± 2.83
Pooled	929	16.53 ± 2.22	25.13 ± 2.44	32.12 ± 2.08	37.46 ± 2.07	41.61 ± 2.08	45.01 ± 2.03	48.08 ± 2.14	51.46 ± 2.83

Table 8. Average values of estimated shell lengths in each annulus group in yaminonishiki form

Annulus group	N	Estimated shell length (mm) ± Standard deviation (unbiased value)								
		r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9
1	0									
2	0									
3	11	17.52 ± 3.33	26.56 ± 2.14	34.13 ± 2.33						
4	67	15.55 ± 2.63	24.72 ± 2.35	32.31 ± 2.37	38.08 ± 2.59					
5	94	15.66 ± 2.15	24.16 ± 2.46	31.89 ± 2.31	37.93 ± 2.43	42.44 ± 2.39				
6	53	16.15 ± 2.20	24.52 ± 2.41	31.72 ± 2.22	37.66 ± 2.36	42.28 ± 2.06	45.85 ± 2.29			
7	29	15.77 ± 2.31	23.88 ± 2.32	31.34 ± 1.96	37.15 ± 1.76	41.85 ± 1.69	45.67 ± 1.85	48.43 ± 2.04		
8	3	17.54 ± 4.76	25.24 ± 3.78	32.27 ± 4.31	37.03 ± 2.94	41.86 ± 1.59	45.50 ± 1.95	49.56 ± 1.58	52.66 ± 1.34	
9	1	15.62	23.77	30.19	34.60	38.92	41.89	44.86	47.45	48.15
Pooled	258	15.85 ± 2.42	24.46 ± 2.44	32.00 ± 2.33	37.80 ± 2.40	42.27 ± 2.19	45.73 ± 2.15	48.43 ± 2.08	51.36 ± 2.83	48.15

Subsequently, Walford growth transformation formulae were calculated with all estimated shell length values at the annulus formation by using the methods of Yokogawa (1992a, 1992b). High correlation was recognized between L_t (shell length at age t) and L_{t+1} (shell length at age t+1); in both the forms, the following two formulae were given. This suggested that the awajichihiro and yaminonishiki forms both fit with Walford growth transformation and von Bertalanffy growth formula.

Awajichihiro form $L_{t+1} = 0.783 L_t + 12.34$ ($r = 0.974$, $N = 3291$)
Yaminonishiki form $L_{t+1} = 0.806 L_t + 11.96$ ($r = 0.982$, $N = 1066$)

The von Bertalanffy growth formulae conducted from the parameters of these Walford growth transformation formulae were as follows.

Awajichihiro form $L_t = 56.95\ (1 - e^{-0.244\ (t + 0.404)})$
Yaminonishiki form $L_t = 61.64\ (1 - e^{-0.216\ (t + 0.378)})$

For this study, ***t*** tests were performed to compare the parameters of the Walford growth transformation formulae of the awajichihiro and yaminonishiki forms, so that ultimately the differences between the two forms could be examined. Based on the test results, that t=3.873 (P=0.0001) for the inclination, and t=0.057 (P=0.9546) for the intercept, it was determined that the inclination differed significantly from each other at the 0.1% level, and the intercept did not differ. Namely, there was no difference in early growth of the awajichihiro and yaminonishiki forms, but growth rates of the two forms significantly differed from each other, with that of the yaminonishiki form being greater.

As a confirmation of that, the shell length limit value (L_∞) in the von Bertalanffy growth formula of the yaminonishiki form was about 5 mm larger than that of the awajichihiro form, inferring that the yaminonishiki form can grow larger than the awajichihiro form. Regarding the shell length frequency distributions, proportions of the aged individuals are higher in the yaminonishiki form (Table 8); calculations of average age of all the specimens examined resulted in 4.549 and 5.136 in the awajichihiro and yaminonishiki forms, respectively. This suggests that longevity of the yaminonishiki form is greater; also, this might be connected with the better growth rate of the yaminonishiki form as mentioned earlier.

In contemplating reasons why the growth rate of the yaminonishiki form is greater than that of the awajichihiro form, assuming that the two forms are distinct subspecies (Habe and Kosuge, 1967; Kira, 1972; Habe, 1977; Habe and Okutani, 1985) as introduced in Chapter 1, growth rate can be described as unique biological characteristics to each "subspecies."

On the other hand, another probability can be hypothesized as follows.

In the case of mollusks with shells, the shells are created mainly by the secretion of calcium carbonate (Wada, 1994; Suzuki ***et al.***, 1996; Kobayashi, 1996). As reported in Chapter 2 and Hayami (1985), the yaminonishiki form had relatively less shell weight (volume) owing to the weakness of the radial costae; therefore, the amount of the calcium carbonate required for the shell creation could be less.

Here, suppose that the creative abilities of the shells in the awajichihiro and yaminonishiki forms were parallel. The yaminonishiki form could save the calcium carbonate for the shell creation, and the surplus calcium carbonate also could be used for the shell growth, resulting in excellent growth. Such a hypothesis that the differences in relative shell weight and growth rate between the awajichihiro and yaminonishiki forms are connected provides a rational explanation. In such a case, the difference in the growth rate indirectly expresses the morphological difference between the two forms.

Hayami (1984) examined the growth in two forms of blistered scallop, ***Cryptopecten vesiculosus*** (strong and weak costa forms) by annulus analysis. His data indicated that growth rate of the weak costa form clearly excelled that of the strong costae form. This is quite consistent with the phenomenon that growth rate of the weak costal yaminonishiki form was greater, and can support the hypothesis that strength of the radial costae affects the growth rate.

The growth analysis in the present study revealed that the growth of the two forms in ***Volachlamys hirasei*** both corresponded well with von Bertalanffy growth formula, and the growth rate of the yaminonishiki form was greater than that of the awajichihiro form. This contradicts the hypothesis given in Chapter 3 that the two forms are identical reproductive populations; but on the other hand, it may reflect the differences observed in the external morphology between the two forms reported in Chapter 2.

Before concluding the taxonomic treatment of the awajichihiro and yaminonishiki forms, further examination of biological information remained. In the following chapter, a study from a unique standpoint will be described.

Chapter 5 Parasitism of awajichihiro and yaminonishiki forms by a pea crab, *Pinnotheres sinenis*

A pea crab, ***Pinnotheres sinenis*** (Fig. 16) is a small species belonging to family Pinnotheridae. It has a specialized ecological characteristic of parasitism on the mantle of bivalve shells such as the Pacific oyster, ***Crassostrea gigas***, the Japanese common clam, ***Ruditapes philippinarum***, the hard-shelled mussel, ***Mytilus coruscus***, the common blue mussel, ***Mytilus galloprovincialis***, the pearl oyster, ***Pinctada***

Fig. 16. A pea crab ***Pinnotheres sinenis***
(Upper individual: Female, Lower individual: Male)

fucata, Farrer's scallop, ***Chlamys farreri*** and the Japanese cockle, ***Fulvia mutica*** (Sakai, 1974; Takeda, 1975; Sakaguchi, 1979; Miyake, 1983). It's quite possible that people have taken a bite of this species when eating the Japanese common clams. In Japan, this species is widely distributed from southeastern Hokkaido to the whole of Honshu and the western coast of Kyushu (Konishi, 1996). It prefers inland waters rather than open waters. It is reported that the pea crab is mainly parasitic on ***Mytilus galloprovincialis*** in the Seto Inland Sea (Ueda and Kohno, 1987). This species has retrogressive morphological characteristics adapted for the parasitic life, morphological specialization is recognized even in its larval stages (Yastuzuka and Iwasaki, 1979).

During the morphological and ecological examinations to reveal the relationship between the two forms of Hirase's scallop, ***Volachlamys hirasei***, pea crabs, ***Pinnotheres sinenis***, parasitizing on ***V. hirasei*** were commonly observed. Although ***P. sinenis*** has wide selectivity for its host (Konishi, 1996), it has never been reported that ***P. sinenis*** uses ***V. hirasei*** as its host (Ueda and Kohno, 1987; Konishi, 1996). Therefore, this chapter reports on the relationship between ***P. sinenis*** and ***V. hirasei***, including the selectivity of ***P. sinenis*** for the two forms of ***V. hirasei***.

Materials and methods

Specimens for this chapter were the same ones examined in Chapters 2–4, being caught via trawl in the Bisan-Seto waters of Kagawa from April, 1989 to March, 1990. The specimens were classified into the awajichihiro and yaminonishiki forms with the criteria described in Chapter 2, and condition factor (CF) was calculated with the formula in Chapter 3.

Results and discussion

Table 9 shows monthly appearance of ***Pinnotheres sinenis***. ***P. sinenis*** appeared almost year round both in the awajichihiro and yaminonishiki forms. In particular, ***P. sinenis*** tended to be frequent in the autumn and winter seasons (Table 9). Ueda

Table 9. Appearance of ***Pinnotheres sinenis*** in awajichihiro and yaminonishiki forms

	Awajichihiro form					Yaminonishiki form					
	Na[*1]	***Pinnotheres sinenis***				Ny[*1]	***Pinnotheres sinenis***				χ^2ay[*4]
		Male[*2]	Female[*2]	Pooled[*2]	χ^2mf[*3]		Male[*2]	Female[*2]	Pooled[*2]	χ^2mf[*3]	
April	58	10.3	6.9	17.2	0.400	20	5.0	15.0	20.0	0.063	0.063
May	221	9.0 ★	8.6 ★	17.6	0.026	49	2.0	2.0	4.1	4.860 *	4.860 *
June	46	4.3	15.2	19.6	2.778	14	7.1	14.3	21.4	0.019	0.019
July	69	1.4	21.7	23.2	12.250 ***	17	11.8	0.0	11.8	0.850	0.850
August	76	7.8	11.7	19.5	0.600	12	0.0	0.0	0.0	2.090	2.090
September	93	18.3	12.9 ★	31.2	0.862	21	14.3	9.5	23.8	0.312	0.312
October	45	15.6	15.6	31.1	0.000	20	25.0 ★	10.0	35.0	0.065	0.065
November	55	18.2	23.6	41.8 ☆	0.391	13	15.4	7.7	23.1	0.966	0.966
December	37	16.2 ★	8.1	24.3	1.000	25	12.0	16.0	28.0	0.078	0.078
January	72	30.6 ★★	16.7	47.2	2.941	19	5.3	15.8	21.1	2.465	2.465
February	76	18.4 ★	13.2	31.6 ☆	0.667	18	22.2 ★★	16.7	38.9	0.236	0.236
March	81	23.5 ★★★	12.3	35.8 ☆☆☆	2.793	30	13.3 ★	23.3	36.7	0.005	0.005
Total	929	14.0	13.0	27.0	0.326	258	10.5	10.9	21.3	2.528	2.528

[*1] Na: Individual numbers of awajichihiro form, Ny: Individual numbers of yanimonishiki form

[*2] Appearance frequency (%) of ***Pinnotheres sinenis*** (male, female, pooled)

[*3] Chi-square test for difference in appearance of male and female ***Pinnotheres sinenis*** (*** significant at 0.1% level)

[*4] Chi-square test for difference in appearance of ***Pinnotheres sinenis*** in awajichihiro and yaminonishiki forms（* significant at 5% level）

★ Case that two males or females are parasitic on a single host (★ number indicates case number)

☆ Case that one male and one female are parasitic on a single host（☆ number indicates case number) and Kohno (1987) also reported considerable seasonal changes in appearance of this species.

The small body of male ***P. sinenis*** (Fig. 16) enables it to enter and exit the hosts freely, while the larger body of female ***P. sinenis*** (Fig. 16) does not permit it to exit the host once it settles itself in the host (Takeda, 1975). Morphologically, pleopods of the males have long hairs to swim, while the swimming hairs do not develop on those of the females except in the immature swimming stage (Miyake, 1983).

If adult females are parasitic permanently on single hosts, the appearance of the females should be stable throughout the season. This does not explain the seasonal changes of the appearance (Table 9). Perhaps the females also may move in and out of their hosts freely like the males, or perhaps the mortality of the females changes by season. The former probability is more likely because the host ***V. hirasei*** can open its valves wide with just a small action of its hinge.

Chi-square tests to examine differences in frequency of male and female ***P. sinenis*** showed no significance except in the data of the awajichihiro form in July (Table 9), suggesting that both sexes of ***P. sinenis*** do not select either forms. Also, the chi-square tests to examine differences in frequency of ***P. sinenis*** in the awajichihiro and yaminonishiki forms showed no significance except in the data in May (Table 9), suggesting that ***P. sinenis*** do not select either forms.

Some cases that multiple individuals of ***P. sinenis*** were parasitic on a single host were observed; in particular, two males were remarkably parasitic together (Table 9). Those cases concentrated in the winter from January to March. In another case, one male and one female being parasitic on a single host were observed 4 times in total in February and March (Table 9).

Haines ***et al.*** (1994) examined ecology of a kind of pea crab, ***Pinnotheres pisum*** from southern England, which is parasitic on mussels, ***Mytilus edulis***. They reported the considerable appearance of male/female pairs, inferring that the species mates inside the hosts. In addition, Soong (1997) examined ecology of another kind of pea crab, ***Pinnotheres tsingtaoensis*** from Taiwan, which is parasitic on the bivalve shell ***Saguinolaria acuta***, and also strongly suggested the probability that the pea crabs mate inside the host shells. He hypothesized that the free-living males visited the permanent resident females and that they mated there.

These reports suggest that the pea crabs mate when male/female pairs occur in single hosts. In the case of ***Pinnotheres sinenis***, the period from January to March (when male/female pairs and multiple individuals frequently appear in single hosts) is expected to be the mating season. Cases of multiple males appearing in single hosts increased in this season, suggesting that males actively move among the hosts to seek females to mate. Berrying (egg-bearing) season of ***P. sinenis*** is described as July (Miyake, 1983), and from July to October (the most being in September) (Sakaguchi, 1979). Considering, then, when berrying season occurs, it might be reasonable to regard ***P. sinenis*** mating season as winter, from January to March, although the period of time from fertilization to hatching of zoeal larvae is uncertain. In the case of ***Pinnotheres pisum*** from England, mating (fertilization) period and berrying period are expected to be from February to May and from April to October, respectively (Haines ***et al.***, 1994), an interval between the two periods corresponding well with that in the hypothesis in ***P. sinenis***.

Table 10 shows condition factors (CF) of ***Volachlamys hirasei*** by parasitism and non-parasitism of ***Pinnotheres sinenis***. The CF values of ***V. hirasei*** do not differ in the same month and indicate very similar seasonal changes, that is, CF are high from winter to spring and low from summer to autumn (see Chapter 3).

The CF values of the hosts by parasitism of female ***P. sinenis*** tended to be lower than those by parasitism of the males (Table 10). Also, the CF values of ***V. hirasei*** by parasitism of ***P. sinenis*** tended to be lower than those by non-parasitism (Table 10).

Parasitic ecology of the pea crabs has not been well revealed; however, the parasitism of the hosts by the pea crabs are expressed as "parasitic disease" or "morbidness" in the fisheries (Sakaguchi, 1979). Mantle of the hosts and its marginal region are influenced by the parasitism and growth of shell margin stops. Also, an abnormal secretion of an organic substance is observed overall inside the shells; the soft body is emaciated and the hosts are inclined to die with sudden changes of habitat conditions (Sakaguchi, 1979). In such cases where the pea crabs are parasitic on Japanese common clam, ***Ruditapes philippinarum***, they damage gills of the

Table 10. Condition factor of ***Volachlamys hirasei*** by parasitism and non-parasitism of ***Pinnotheres sinenis***

	Awajichihiro form					Yaminonishiki form				
	Na[*1]	***Pinnotheres sinenis***				Ny[*1]	***Pinnotheres sinenis***			
		Male[*2]	Female[*2]	Pooled[*2]	No parasite[*3]		Male[*2]	Female[*2]	Pooled[*2]	No parasite[*3]
April	58	4.383	5.264	4.736	4.957	20	4.523	4.858	4.774	5.237
May	221	4.516	4.631	4.572	4.689	49	5.219	3.090	4.155	4.847
June	46	4.837	4.644	4.687	4.950	14	4.115	2.887	3.297	4.931
July	69	3.968	3.026	3.085	3.283	17	3.043	—	3.043	3.187
August	76	3.549	3.236	3.361	3.990	12	—	—	—	4.051
September	93	1.498	1.563	1.525	1.707	21	1.616	1.453	1.551	1.755
October	45	2.745	2.544	2.645	2.825	20	2.519	2.991	2.654	3.049
November	55	2.776	2.359	2.540	3.363	13	2.704	3.023	2.811	2.905
December	37	5.030	4.663	4.908	4.986	25	4.936	4.692	4.797	4.748
January	72	5.903	5.297	5.689	5.747	19	5.051	5.827	5.633	5.872
February	76	5.788	5.722	5.761	6.198	18	5.122	5.530	5.297	6.134
March	81	5.458	5.397	5.437	5.837	30	5.787	5.116	5.360	5.745

[*1] Na: Individual numbers of awajichihiro form, Ny: Individual numbers of yanimonishiki form

[*2] Average values of condition factor of individuals with parasitism by ***Pinnotheres sinenis***

[*3] Average values of condition factor of individuals without parasitism by ***Pinnotheres sinenis***

host shells and influence fatness of the hosts; the fatness of the "morbid shells" is about 75% that of the non-parasitized shells (Sakaguchi, 1979). In the case of the cultured pearl oyster, ***Pinctada fucata***, 70–90% of operated shells were killed with the parasitism of the pea crabs in a pearl nursery in the Seto Inland Sea (Sakaguchi, 1979).

The information obviously indicates that ***P. sinenis*** greatly stresses the hosts, and conditions of the parasitized hosts strongly infers that ***P. sinenis*** appropriates food and nutrition which should be for the hosts, perhaps even utilizing some portion of the hosts' body for food. This also can correspond to the case of ***Volachlamys hirasei***. CF decreases owing to stress caused by the parasitism of ***P. sinenis***; in particular, parasitism by the females, whose body sizes are larger than those of the males (Fig. 16), can influence the hosts more greatly.

Thus, regarding a relationship between ***V. hirasei*** and ***P. sinenis***, the results in this chapter suggest a potential that ***P. sinenis*** does not distinguish the awajichihiro and yaminonishiki forms. While the parasitism of ***P. sinenis*** greatly inclines toward the common blue mussel, ***Mytilus galloprovincialis*** in the Seto Inland Sea (Ueda and Kohno, 1987), ***P. sinenis*** has a clear preference to select the hosts (species). These matters might indirectly suggest that the awajichihiro and yaminonishiki forms are the same species.

To conclude the taxonomic treatment of the awajichihiro and yaminonishiki forms, in addition to the morphological and ecological information revealed in earlier chapters, the genetic information should be indispensable as pointed out by Hayami (1985). Thus, the next chapter will genetically examine the two forms.

Chapter 6 Genetic characteristics of awajichihiro and yaminonishiki forms

To reveal the relationship between the two forms (awajichihiro and yaminonishiki) of ***Volachlamys hirasei***, Chapter 2 examined the differences in the external morphology of the two forms, indicating considerable morphological differences between the two forms. Subsequently, Chapter 3 examined the internal characters of the two forms and their seasonal changes, reporting no difference in the internal morphology between the two forms and considerable similarity in their seasonal change patterns. Further, Chapter 4 examined the age and growth of the two forms, and reported that there was a significant difference in the growth rate between the two forms, namely growth of the yaminonishiki form was greater than that of the awajichihiro form. Additionally, Chapter 5 examined the parasitism by a pea crab, **Pinnotheres sinenis** on the two forms, and suggested that **P. sinenis**, which has strong selectivity for the hosts, did not distinguish the two forms.

This chapter examines the genetic structure of the two forms by isozyme analysis to resolve the relationship problem, and will present an analytical discussion based on all the biological data obtained for the two forms.

Materials and methods

Specimens for this chapter were caught via trawl in the Bisan-Seto waters of Kagawa on March 6, 1995. The specimens were classified into the awajichihiro and yaminonishiki forms with the criteria in Chapter 2. The number of examined specimens totaled 168, consisting of 104 awajichihiro and 64 yaminonishiki forms.

The collected specimens were immediately frozen to −80 ℃ using a freezer, and thereafter preserved until the genetic experiments. Isozymes detected by electrophoresis were used as genetic markers; extraction from the digestive diverticulum and adductor muscle was used for the electrophoresis. Methods of the experiments and transcription of loci and alleles totally followed Yokogawa (1996b). For detection of esterase (***EST-1**** locus) in the extraction from the digestive diverticulum, the thin polyacryl-amide-gel electrophoresis after Taniguchi and Tashima (1978) was introduced.

Results

Following the electrophoresis, 15 enzymes and 1 non-enzymic protein were detected, with 24 presumed loci (Table 11).

Initially, fitness of the allelic frequencies in polymorphic loci, according to Hardy-Weinberg equilibrium, was examined by chi-square tests in the awajichihiro form, yaminonishiki form and both the forms pooled (hereafter called form mixture) (Table 12). Because no χ^2 values were significant at the 5% level in the awajichihiro and yaminonishiki forms and the form mixture, all corresponded well with the Hardy-Weinberg equilibrium. Here, both the awajichihiro and yaminonishiki forms were regarded as having originated from simple Mendelian populations, while the form mixture was regarded similarly, not proving that the two forms are genetically independent populations.

Values to indicate the genetic features, including average allele numbers per locus, rate of polymorphic loci and average heterozygosity (average rate of heterozygous loci per individual) of the awajichihiro and yaminonishiki forms and the form mixture, are shown in Table 13. It is well known that genetic variability in the mollusks is extremely higher than that in other animals (Fujio ***et al.***, 1983; Crow, 1989). In case of the two forms of ***Volachlamys hirasei***, although the rates

Table 11. Enzymes, protein and tissues examined

Enzyme or protein name	Enzyme number	Locus	Subunit structure	Tissue
Aspartate aminotransferase	2.6.1.1	**_AAT-1*_**	Dimeric	Adductor muscle
		AAT-2*		Adductor muscle
Acid phosphatase	3.1.3.2	**_ACP-1*_**		Digestive diverticulum
		ACP-2*		Digestive diverticulum
Catalase	1.11.1.6	**_CAT*_**		Adductor muscle
Creatine kinase	1.6.99.7	**_CK-1*_**		Adductor muscle
		_CK-2*_	Monomeric	Adductor muscle
Esterase	3.1.1.-	**_EST-1*_**	Monomeric	Digestive diverticulum
		EST-2*		Adductor muscle
Glycerol-3-phosphate dehydrogenase	1.1.1.8	**_G3PDH*_**	Monomeric	Adductor muscle
Glucose-6-phosphate isomerase	5.3.1.9	**_GPI*_**	Dimeric	Adductor muscle
Isocitrate dehydrogenase (NADP$^+$)	1.1.1.42	**_IDHP-1*_**		Adductor muscle
		IDHP-2*	Dimeric	Adductor muscle
Leucine aminopeptidase	3.4.11.1	**_LAP*_**	Monomeric	Digestive diverticulum
Malate dehydrogenase	1.1.1.37	**_MDH-1*_**	Dimeric	Adductor muscle
		MDH-2*		Adductor muscle
Malic enzyme (NADP$^+$)	1.1.1.40	**_MEP-1*_**	Tetrameric	Digestive diverticulum
		MEP-2*	Tetrameric	Digestive diverticulum
Mannose-6-phosphate isomerase	5.3.1.8	**_MPI*_**	Monomeric	Adductor muscle
Phosphogluconate dehydrogenase	1.1.1.44	**_PGDH*_**	Dimeric	Adductor muscle
Phosphoglucomutase	5.4.2.2	**_PGM*_**	Monomeric	Adductor muscle
General protein		**_PROT-1*_**		Adductor muscle
		PROT-2*		Adductor muscle
Superoxide dismutase	1.15.1.1	**_SOD*_**		Digestive diverticulum

of the polymorphic loci are consistent with the standard level of general mollusks (Fujio ***et al.***, 1983), the average heterozygosities are less than the standard level (Fujio ***et al.***, 1983; Crow, 1989), indicating somewhat less variability than the other mollusks.

The resulting values to indicate the genetic features were quite similar to one another in the awajichihiro and yaminonishiki forms and the form mixture (Table 13); they did not prove that the awajichihiro and yaminonishiki forms are genetically independent populations, as in the case that fitness of the allelic frequencies for Hardy-Weinberg equilibrium was examined.

Selected electrophoretograms in some significant loci are illustrated in Figure 17, and allelic frequencies of the awajichihiro and yaminonishiki forms are shown in Table 14. Some of these loci and alleles are described as follows.

Glycerol-3-phosphate dehydrogenase (G3PDH) indicated an activity in the adductor muscle; one locus of **_G3PDH*_** was presumed in the anodal zone of the

Table 12. Chi-square tests of fitness for Hardy-Weinberg equilibrium in groups of awajichihiro form, yaminonishiki form and both forms pooled

	Awajichihiro		Yaminonishiki		Pooled	
	χ^2	P[*1]	χ^2	P[*1]	χ^2	P[*1]
AAT-1*	0.003	0.956	0.006	0.938	0.007	0.933
CK-2*	0.014	0.906			0.012	0.913
EST-1*	4.858	0.562	7.652	0.265	3.552	0.737
G3PDH*	0.083	0.994	0.120	0.989	0.197	0.978
GPI*	0.587	0.899	0.214	0.975	0.784	0.853
IDHP-2*	1.040	0.792	1.945	0.163	0.209	0.976
LAP*	1.280	0.734	0.327	0.955	1.402	0.705
MDH-1*	3.113	0.375	3.249	0.355	5.370	0.147
MEP-1*	0.168	0.682	3.452	0.063	1.333	0.248
MEP-2*	0.112	0.990	0.022	0.999	0.127	0.988
MPI*	0.546	0.644	1.348	0.246	1.758	0.185
PGDH*	0.213	0.975	0.037	0.998	0.230	0.999
PGM*	2.666	0.446	1.272	0.736	2.589	0.460

[*1] Risk percentage for chi-square value

Table 13. Values to indicate genetic features in groups of awajichihiro form, yaminonishiki form and both forms pooled

		Awajichihiro	Yaminonishiki	Pooled
Alleles/Locus		1.958	1.875	2.000
P*		0.333	0.333	0.333
P		0.208	0.167	0.208
P+P*		0.542	0.500	0.542
Average Heterozygosity	Ho	0.084	0.086	0.085
	He	0.084	0.083	0.084
	Ho/He	1.001	1.038	1.013

P* : Polymorphism less than 0.95 P : Polymorphism over 0.95

Ho : Observed heterozygosity He : Expected heterozygosity

electrophoretogram. The ***G3PDH**** locus of both the forms indicated polymorphism with 3 loci (Table 14), and showed monomeric subunit structure in which heterozygotes display 2 bands in the electrophoretogram (Fig. 17). Frequency of each allele was very similar to each other in the two forms, not indicating any significant differences in the allelic frequencies (Table 14).

Isocitrate dehydrogenase (IDHP) indicated high activity in the adductor muscle, and two band zones appeared in the anodal zone. Among those, the zone with high mobility was defined as the ***IDHP-1**** locus and that with low mobility was defined as the ***IDHP-2**** locus (Fig. 17). The ***IDHP-2**** locus showed polymorphism

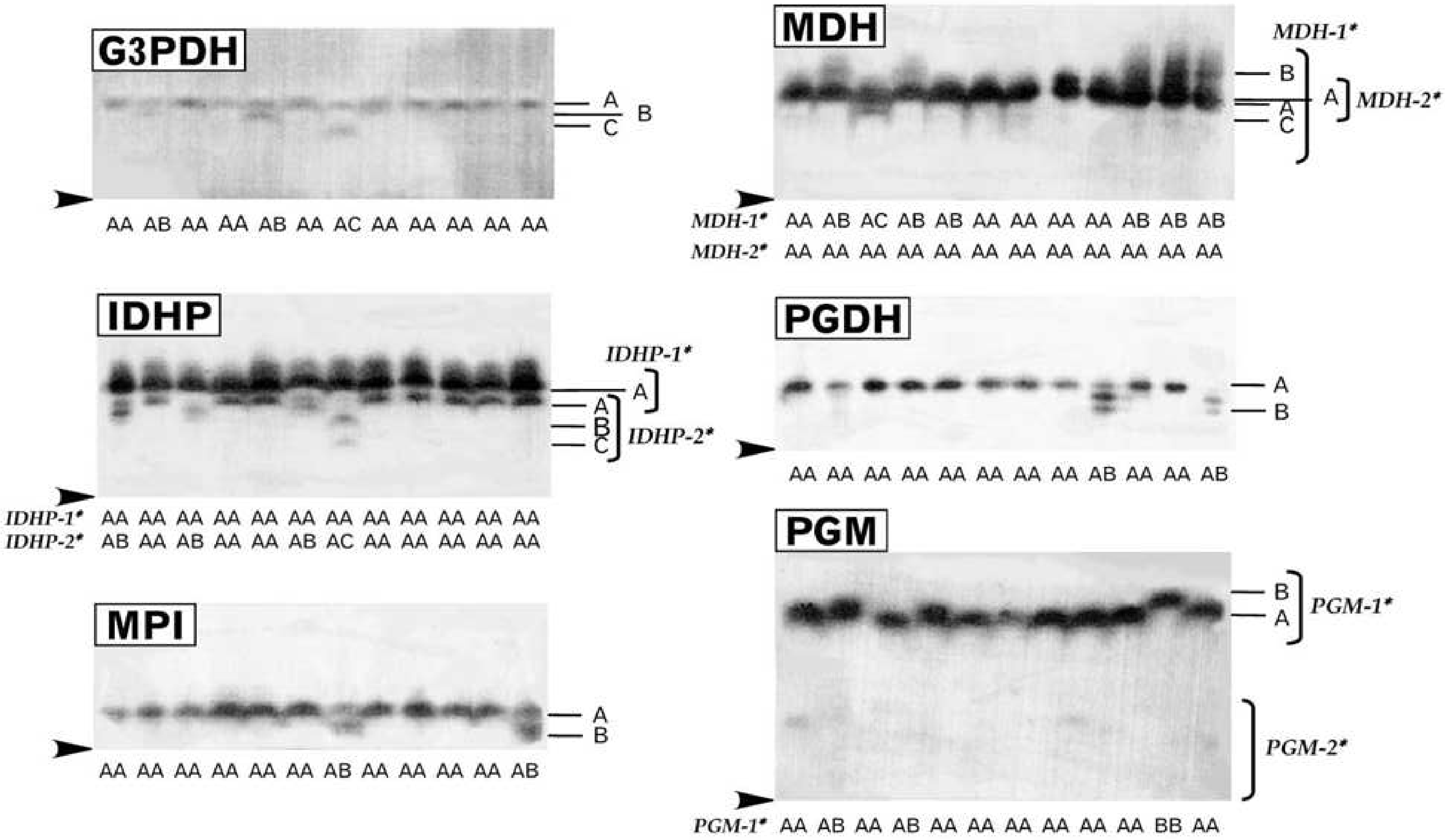

Fig. 17. Electrophoretograms at some remarkable enzymes in ***Volachlamys hirasei***. Alleles are symbolized as capital alphabet signs by Table 14.

Table 14. Allelic frequencies of awajichihiro and yaminonishiki forms with χ^2 heterogeneities between both frequencies

Locus	Allele	Frequency		χ^2 hetero	d.f.[*1]	P[*2]
		Awajichihiro	Yaminonishiki			
AAT-1*	*120 B	0.005	0.011	0.303	1	0.582
	*100 A	0.995	0.989			
AAT-2*	*0 A	1.000	1.000	—		
ACP-1*	*100 A	1.000	1.000	—		
ACP-2*	*100 A	1.000	1.000	—		
CAT*	*-100 A	1.000	1.000	—		
CK-1*	*100 A	1.000	1.000	—		
CK-2*	*-75 B	0.014	0.000	0.450	1	0.502
	*-100 A	0.986	1.000			
EST-1*	*110 C	0.019	0.016	0.222	3	0.974
	*105 B	0.117	0.113			
	*100 A	0.825	0.823			
	*90 D	0.039	0.048			
EST-2*	*100 A	1.000	1.000	—		
G3PDH*	*100 A	0.969	0.956	0.301	2	0.860
	*90 B	0.025	0.035			
	*75 C	0.006	0.009			
GPI*	*115 C	0.036	0.023	0.475	2	0.789
	*100 A	0.928	0.945			
	*85 B	0.036	0.031			
IDHP-1*	*100 A	1.000	1.000	—		
IDHP-2*	*100 A	0.856	0.852	0.708	2	0.702
	*80 B	0.139	0.148			
	*55 C	0.005	0.000			
LAP*	*110 C	0.105	0.063	0.575	2	0.750
	*100 A	0.842	0.875			
	*90 B	0.053	0.063			
MDH-1*	*120 B	0.134	0.172	0.872	2	0.647
	*100 A	0.809	0.773			
	*70 C	0.057	0.055			
MDH-2*	*100 A	1.000	1.000	—		
MEP-1*	*100 A	0.940	0.930	0.074	1	0.786
	*90 B	0.060	0.070			
MEP-2*	*130 B	0.023	0.010	0.565	2	0.754
	*100 A	0.965	0.979			
	*65 C	0.012	0.010			
MPI*	*100 A	0.927	0.909	0.270	1	0.603
	*50 B	0.073	0.091			
PGDH*	*150 D	0.005	0.000	3.661	3	0.300
	*100 A	0.957	0.977			
	*65 B	0.038	0.016			
	*40 C	0.000	0.008			
PGM*	*110 B	0.094	0.094	0.303	2	0.613
	*100 A	0.878	0.867			
	*90 C	0.028	0.039			
PROT-1*	*100 A	1.000	1.000	—		
PROT-2*	*100 A	1.000	1.000	—		
SOD*	*100 A	1.000	1.000	—		

[*1] Degree of freedom [*2] Risk percentage for chi-square value

with dimeric subunit structure, including 3 alleles of ****100***, ****80*** and ****55*** in the awajichihiro form, and 2 alleles of ****100*** and ****80*** in the yaminonishiki form (Fig. 17, Table 14). At the ***IDHP-2**** locus, the allelic frequencies of the two forms were very similar to each other, except that the frequency of the ****55*** allele was 0 in the yaminonishiki form; any significant differences were not recognized in the allelic frequencies between the two forms (Table 14).

Malate dehydrogenase (MDH) showed stable activity in the adductor muscle; two overlapped band zones were observed in the anodal zone.

Among those, the polymorphic band zone with dimeric subunit structure was defined as the ***MDH-1**** locus, while the other monomorphic band zone was defined as the ***MDH-2**** locus (Fig. 17). At the ***MDH-1**** locus, 3 alleles of ****120***, ****100*** and ****70*** were presumed in both the forms, notably mobility of the ****100*** allele, which is the main allele in both the forms, was almost parallel with that of the ****100*** allele at the ***MDH-2**** locus (Fig. 17). The allelic frequencies of the two forms at the ***MDH-1**** locus were similar to each other; any significant differences were not recognized between the two forms (Table 14).

Mannose-6-phosphate isomerase (MPI) indicated the activity in the adductor muscle; a band zone appeared near to the origin in the anodal zone of the electrophoretogram, and a single locus of ***MPI**** was presumed. The ***MPI**** locus showed polymorphism with monomeric subunit structure, including 2 alleles of ****100*** and ****55*** in both the forms (Fig. 17, Table 14). The allelic frequencies of the two forms were close to each other; any significant differences were not recognized (Table 14).

Phosphogluconate dehydrogenase (PGDH) indicated high activity in the adductor muscle; a single locus of ***PGDH**** was presumed in the anodal zone. The ***IDHP-2**** locus showed polymorphism including the alleles of ****150***, ****100*** and ****65*** in the awajichihiro form, and those of ****100***, ****65*** and ****40*** in the yaminonishiki form (Table 14), with dimeric subunit structure in which the heterozygotes display 3 bands (Fig. 17). The allelic frequencies of the two forms were very similar to each other, except that the frequencies of the minor alleles of ****150*** and ****40*** were both 0 in the yaminonishiki and awajichihiro forms, respectively, and any significant differences were not recognized in the allelic frequencies between the two forms (Table 14).

Phosphoglucomutase (PGM) indicated high activity in the adductor muscle; a band zone that appeared far from the origin in the anodal zone was defined as the ***PGM**** (Fig. 17). While presence of another band zone was recognized nearer to the origin, it was not used for the genetic analysis because the bands could not be read exactly in many individuals, owing to unstableness of the enzyme activity under the experimental method in the present study. At the ***PGM**** locus, 3 alleles of ***110**, ***100** and ***90** were presumed in both the forms, and the allelic frequencies of the two forms were very close to each other; any significant differences were not recognized (Table 14).

At all the remaining polymorphic loci, any significant differences also were not recognized between the allelic frequencies of the two forms, and the other monomorphic loci of the two forms were totally occupied by the same alleles (Table 14).

The genetic distance (D value) between the two forms, calculated from isozymic allele frequencies after Nei (1972) was 0.0002, being the level lower than the local population or within the identical population (Nei, 1990).

In addition, linkage between the strength of the radial costae and the genotypes at the isozymic loci, and that between the shell coloration and the genotypes were examined individually; however, any linkage between such morphological characteristics and the genotypes was not detected at any loci.

Discussion

In the two forms of ***Volachlamys hirasei*** examined in the present study, the electrophoretogram of glycerol-3-phosphate dehydrogenase (G3PDH) showed monomeric subunit structure (Fig. 17). Although G3PDH shows dimeric subunit structure in the fishes and most of the shells (Fujio, 1984; Japan Fisheries Resource Conservation Association, 1989), it unusually shows the clear monomeric subunit structure in a pectinid shell of the yesso scallop, ***Patinopecten yessoensis*** (Yamanaka and Fujio, 1983; Fujio, 1984; Kijima ***et al.***, 1984). However this is not a universal feature throughout the species in family Pectinidae, because a dimeric band pattern is observed in the other pectinid shell of the Japanese baking scallop, ***Pecten albicans*** (Kijima, 1989). It is interesting that ***V. hirasei*** shows the monomeric subunit structure to be the same as ***P. yessoensis***, because such subunit structure in the isozyme may reflect phylogenetic systematics of livings (Yokogawa, 1996c). Therefore, further isozymic examinations on the many remaining species in this family will reveal the relationship between the subunit structure and the phylogenetic systematics.

As for the genetic features of the awajichihiro and yaminonishiki forms, evidence that the two forms are genetically independent populations was not recognized by the results of fitness for Hardy-Weinberg equilibrium (Table 12) and the values to indicate the genetic features (Table 14). Moreover, there were no differences in the allelic frequencies between the two forms, and the genetic distance between the two forms was the level lower than the local population or within the identical population.

These results by genetic analysis can support a hypothesis that the awajichihiro and yaminonishiki forms are the identical reproductive population, namely the same species, and the differences observed in the external morphology (Sato, 1975; Hayami, 1985; also see Chapter 2) are intraspecific morphological variation. This is supported strongly by the facts that the two forms inhabit sympatrically (Sato, 1975; Hayami, 1985; also see Chapter 2), and they have the same reproductive cycles (see Chapter 3). On the other hand, there is an ecological difference of growth the growth rate of the yaminonishiki form is greater than that of the awajichihiro form. This may reflect the morphological difference between the two forms (see Chapter 4).

Assuming that the differences in the external morphology are the intraspecific morphological variation, two cases are probable as factors to raise the variation, namely variation by environmental factors and that by genetic polymorphism. But the former case is invalid because the two forms inhabit sympatrically (Sato, 1975; Hayami, 1985; also see Chapter 2); consequently, the latter case is strongly suggested. This can be supported by a fact that individual proportions of the awajichihiro and yaminonishiki forms are almost stable throughout the year (see Chapter 2). As with this case, the presence of such morphological variation, just like distinct species, is known in many species of living (Sasaji, 1989).

Thus, the two forms are the same species, and it is regarded that they possess a gene (or genes) that is responsible for the characteristic of the weak or no costae, with a certain frequency in the population. This supports the opinion of Hayami (1985). And, as reported in Chapter 2, while the color pattern compositions of the two forms were considerably different from each other, and there were unique color patterns which did not appear in the other form, this infers that the loci responsible for the coloration and the costae morphology are located sympatrically in a single chromosome.

Because no linkage was found between the isozymic loci examined and such morphological variation, the loci responsible for the morphological characters might be located in different chromosomes from those in which the isozymic loci are located. Although chromosome number of ***V. hirasei*** has not been examined yet, that of the relative pectinid shells are 2n=26−38 (the most frequent at 38) (Beaumont and Gruffydd, 1974; Wada, 1978; Komaru and Wada, 1985; von Brand ***et al.***, 1990; Xiang ***et al.*** 1993), being generally more than that of the other mollusks (Hinegardner, 1974; Nakamura, 1985); therefore, such probability is likely.

On the other hand, even in the case that the locus responsible for a morphological character is co-located with a certain isozymic locus in a single chromosome, the linkage of both loci cannot be detected if there is no genetic polymorphism in the isozymic locus. This probability is also very likely because 11 of 24 loci examined in the present study did not show polymorphism (Table 14).

For inheriting mechanism in the characters of the costae strength and coloration, it is difficult to conduct a concrete model only from the results in the present study. Especially in the radial costae, as Sato (1975) and Hayami (1985) pointed out, strength of the costae is stable in the awajichihiro form, whereas in the yaminonishiki form, it shows continuous variation from no costae (complete smooth shell surface) to intermediately strong costae. This suggests the probability in determination of the radial costae morphology that intermediate morphology appears by incomplete dominance of the alleles, or the radial costae morphology is affected by polygenes, in which the amount of similar genes determines the condition of quantitative characters. To prove the probability and to establish the inheriting model, crossbreeding experiments would be indispensable.

In parallel with the two forms in ***V. hirasei***, the occurrence of two morphological forms are known in a relative pectinid shell, the blistered scallop, ***Cryptopecten vesiculosus***. The strength of the radial costae indicates discontinuous distribution (dimorphism) like in ***V. hirasei*** (Hayami, 1984). The dimorphism in ***C. vesiculosus*** also is regarded as the same genetic polymorphism as ***V. hirasei*** (Hayami, 1984), and Sarashina (1995) confirmed such a hypothesis by genetic analysis. In the pectinid shells, such morphological polymorphism in the radial costae might tend to occur as their biological feature.

Considering the inheriting mechanism of the coloration, it should be linked with the inheritance of the radial costae; further, if a factor of gene recombination is added, the mechanism can be more complicated. While, it is reported that the coloration of the shells unexpectedly shows simple Mendelian inheritance in the pearl oyster species, ***Pinctada fucata martensii*** (Wada and Komaru, 1990; Wada and Komaru, 1991; Wada, 1994), the noble scallop, ***Chlamys nobilis*** (Nanba, 1973; Nanba and Nishiyama, 1975), the Japanese cockle, ***Fulvia mutica*** (Fujiwara, 1995), the Japanese common clam, ***Ruditapes philippinarum*** (Yamamoto and Ohashi, 1993; Kishioka, 1995; Kishioka ***et al.***, 1997) and many other species of mollusks (Asami, 1994; Wada and Komaru, 1996). Therefore, in the case of ***V. hirasei***, it may not be so complicated; however, further studies, such as the crossbreeding experiments, are necessary hereafter.

Thus, the examination in this chapter revealed genetic uniformity of the two forms of ***Volachlamys hirasei***. In addition, the ecological uniformity revealed in Chapter 3 also strongly suggests that the two forms are from a single reproductive population. In conclusion, the two forms are the same species and the morphological differences between the two forms represent the intraspecific morphological variation. Pursuant to the conclusion, the following chapter will discuss the taxonomic treatment of the two forms.

Chapter 7 Taxonomic discussion on awajichihiro and yaminonishiki forms

Because the examinations in earlier chapters accumulated much biological information regarding the two forms (awajichihiro and yaminonishiki) of ***Volachlamys hirasei***, this chapter discusses the taxonomic treatment of the two forms with some other relative living species, based on the biological information.

As mentioned in Chapter 1, the two forms of Hirase's scallop ***Volachlamys hirasei*** traditionally have been treated as subspecies of awajichihiro ***V. hirasei awajiensis*** (strong costae form) and yaminonishiki ***V. hirasei hirasei*** (weak costae form) (Habe and Kosuge, 1967; Kira, 1972; Habe, 1977; Habe and Okutani, 1985). On the other hand, some are of the opinion that the two forms should be treated as the same species based on the view that the two forms are regarded as an intraspecific morphological variation (Abbott and Dance, 1983; Matsukuma, 1986; Bernard ***et al.***, 1993; Kato and Fukuda, 1996).

Because it was concluded by the present analytical studies that the two forms of ***V. hirasei*** represent an intraspecific morphological variation, the former opinion is denied. Consequently, the latter opinion is supported. Although there is no exact criterion to define the subspecies, an opinion insists that the subspecies are populations with definitely distinct geographic distributions, and they do not have reproductive isolation when the geographic barrier is removed (Sasaji, 1989). Following this opinion, it is obvious that the two forms of ***V. hirasei*** are not recognized as being subspecies.

Concerning scientific names of the two forms of ***V. hirasei***, as mentioned in Chapter 1, the species name of ***Pecten awajiensis*** was given to the awajichihiro form by Pilsbry (1905), and that of ***Chlamys hirasei*** was given to the yaminonishiki form by Bavay (1904). Because the present analytical studies concluded that the two forms are the identical species, the description by Bavay (1904), which was published earlier, is the valid name. Consequently, the name of ***Pecten awajiensis*** is its junior synonym. In conclusion, the scientific name of the Hirase's scallop is ***Volachlamys hirasei*** (Bavay).

As for the Japanese names, when the two forms are treated as subspecies, they are separately called awajichihiro and yaminonishiki; however, they should be unified because they were concluded to be the identical species. On the frequencies of the awajichihiro and yaminonishiki forms in the Seto Inland Sea, the former is fairly dominant in the level of about twice of the latter (see Chapter 2); also, the dominance of the awajichihiro form is inferred in the Ariake Sea (Sato, 1975). Consequently, it is proposed to unify the Japanese name of ***Volachlamys hirasei*** as "awajichihiro," which is the dominant form in the population and the symbolic morphology in the species.

On the other hand, the awajichihiro and yaminonishiki forms, which are regarded to be the intraspecific morphological variation, should be taxonomically distinctly treated because the two forms can be completely identified with the strength of the radial costae (Sato, 1975; Hayami, 1985; also see Chapter 2). In the description of ***Chlamys hirasei*** (Bavay, 1904), the author defines an individual of the yaminonishiki form, in which the radial costae are weak and the shell surface is almost smooth, as Var. *β* ***ecostata***. His paper also includes a description of a new species of ***Chlamys ambiguus*** from China, which is similar to the awajichihiro form in appearance. Based on those matters, Hayami (1985) defined the yaminonishiki form as ***Volachlamys hirasei*** var. ***ecostata***. Further, he identified ***Chlamys ambiguus*** with the awajichihiro form, rewrote the specific name, and defined the awajichihiro form as ***V. hirasei*** var. ***ambigua***. These definitions are entirely followed by Okutani ***et al***. (1989).

In the case of the yaminonishiki form, the form name of "Var. *β* ***ecostata"*** in the original description by Bavay (1904) means to be "non-costal," and it is noted in the description that "***costis omnino evanidis"*** (costae entirely disappear). However, the illustrated specimen of Var. *β* ***ecostata*** has the costae which are slightly weaker than those of the type specimen of ***Chlamys hirasei***. This is inconsistent with the complete non-costal individual as shown in Kira (1972). Therefore, this naming would be unreasonable. A further problem would be that this form name is given to just some individuals of the yaminonishiki form, not representing the whole individuals of the yaminonishiki form, which includes much morphological variation. Nevertheless, this form name should be used following the opinion of Hayami (1985) to evaluate the viewpoint of "Variety," which Bavay (1904) initially thought up.

Regarding the form name of the awajichihiro form, Hayami's view that ***Chlamys ambiguus*** is an identical species to ***Volachlamys hirasei***, which was the basis for the form name, is doubtful. According to the description of ***Chlamys ambiguus*** (the specific name was rewritten as ***ambigua*** in Hayami [1985] and Bernard ***et al***. [1993], because the genus name of ***Chlamys*** is female gender), width of the radial costae is rather wide and its number is 13, visually underscoring the difference from ***V. hirasei*** (awajichihiro form) from the Seto Inland Sea examined in the present analytical studies. The radial costae numbers of nearly 1000 individuals from the Seto Inland Sea are 13–21, and individuals with 13 costae occupy only 0.6% of the whole (see Chapter 2). Thus, if species are defined with the difference in the radial costae number, ***C. ambigua*** may be a distinct species from ***V. hirasei***.

The definitions by Hayami (1985) are followed by Okutani ***et al.*** (1989), who described "***Volachlamys hirasei*** (awajichihiro form)" from the Yellow Sea. The described specimen is probably from the identical population (species?) of ***C. ambigua***. In their description, the radial costae number of the illustrated specimen is 13, which is equivalent to that of the type specimen of ***C. ambigua*** (Bavay, 1904). While Zhongyan (2004) described "***Volachlamys hirasei*** (awajichihiro form)" from China, the radial costae number of the illustrated specimen also is 13. Although there can be a certain variation range in the radial costae number, such information suggests that mode of the radial costae number of ***C. ambigua*** is located around 13, significantly differing from ***V. hirasei*** in the specific level.

Regarding the marine life populating the China coast, many species are common to those species that inhabit Japan. However, for such species as the top shell, ***Turbo*** (***Batillus***) ***cornutus*** (Ozawa and Tomida, 1995), the red ark shell, ***Scapharca broughtonii*** (Yokogawa, 1997), and the sea bass, ***Lateolabrax japonicus*** (Yokogawa and Seki, 1995), morphological or genetic analysis revealed that the Chinese species actually was a distinct species from the Japanese one. Those reports suggest necessity of careful examination for Chinese "***Volachlamys hirasei***" (***Chlamys ambigua***).

Regarding the above-discussed reason, the basis to use var. ***ambigua*** as the form name of the awajichihiro form is now doubtful; moreover, there even is a view to regard ***Chlamys ambigua*** by Bavay (1904) as a valid species (Bernard ***et al.***, 1993). Consequently, this form name is inappropriate. Instead, a new name of the awajichihiro form should be proposed, that is ***Volachlamys hirasei*** var. ***awajiensis*** after ***Pecten awajiensis***, which is the species name described by Pilsbry (1905), based on the type specimen of the awajichihiro form from the Seto Inland Sea.

According to the discussion, summary of the Japanese, scientific and form names of ***Volachlamys hirasei*** are as follows.

Hirase's scallop (awajichihiro)	***Volachlamys hirasei*** (Bavay, 1904)
Awajichihiro form	***V. hirasei*** var. ***awajiensis***
Yaminonishiki form	***V. hirasei*** var. ***ecostata***

Hayami (1985) also regarded ***Pecten solaris*** and ***Pecten teilhardi*** from the Bohai Sea (Grabau and King, 1928) as identical species to ***V. hirasei***. Among those, ***Pecten solaris*** Born is the awajichihiro type species. Although Grabau and King

(1928) described the radial costae number as 16 in their text, about 20 costae can be counted in the specimens illustrated there. Further, the shell coloration differs from that of ***V. hirasei***, making it difficult to classify the coloration of the illustrated specimen into any of the ***V. hirasei*** color patterns defined in Chapter 2. In terms of such morphological characteristics as the shell shape, radial costae number and coloration, the species ***Pecten solaris*** is more similar to ***Chlamys pica*** (Reeve), also from China (a photograph and a picture are illustrated in Wang ***et al.*** [1988] and Zheng [1989], respectively), and ***Volachlamys singaporina*** (Sowerby), from Southeast Asia (a photograph is illustrated in Abbott and Dance [1983]), than to ***V. hirasei***, inferring that those species are identical with one another.

On the other hand, regarding the morphology of ***Pecten teilhardi***, the yaminonishiki type species that was newly described by Grabau and King (1928), the left valve of the specimen type in the description shows almost no costae. This species also is described in Wang ***et al.*** (1988) with a clear photograph. Although ***P. teilhardi*** is similar in appearance to the yaminonishiki form in ***V. hirasei***, it has the characteristics of shell length being larger than shell height, and has considerably large auricles.

Here, measurements of the ***P. teilhardi*** specimens illustrated in the references was attempted; the picture and photograph were directly measured and proportions were calculated. Values of (shell height)/(shell length) in the specimen of Grabau and King (1928) and that of Wang ***et al.*** (1988) resulted in 94.2% and 92.9%, respectively. Chapter 2 carefully examined the (shell height)/(shell length) values; their averages of the awajichihiro and yaminonishiki forms resulted in 97.1% and 99.1%, respectively. That is, the shell length tends to be larger than the shell height in the awajichihiro form, while the shell length is almost parallel with the shell height in the yaminonishiki form. The descriptions of Grabau and King (1928) and Wang ***et al.*** (1988) indicate that the shell lengths are much larger than the shell height in ***P. teilhardi***, and therefore considerably different from the morphology of the yaminonishiki form in ***V. hirasei***.

Also, values of (auricle width)/(shell length) in the specimen of Grabau and King (1928) and that of Wang ***et al.*** (1988) resulted in 77.6% and 84.0%, respectively. Both are extremely larger values than those calculated for the yaminonishiki form, of which the average value is 65.5% (see Chapter 2). Further, regarding coloration, although the coloration of the specimen in Wang ***et al.*** (1988) can be classified into color pattern 5 (brown), it is difficult to classify the left valve coloration of the specimen illustrated in Grabau and King (1928) into any of the color patterns defined in Chapter 2. If classification were forced, it would be color pattern 2; however, this pattern appeared only in the awajichihiro form and never appeared in the yaminonishiki form (see Chapter 2).

In conclusion, the morphology of ***P. teilhardi*** from China considerably differs from that of the yaminonishiki form in ***V. hirasei***. Therefore, the opinion of Hayami (1985) that identifies ***P. teilhardi*** with ***V. hirasei*** cannot be supported; however, as confirmation, genetic examination should be carried out.

The distribution range of ***V. hirasei*** at present is thought to be from the Seto Inland Sea to China (Habe and Kosuge, 1967), from the Kii Peninsula to Kyushu, Southeast Asia (Matsukuma, 1986), from southeastern Japan to the East China Sea, and the Yellow Sea (Okutani ***et al.***, 1989). The basis for the cited distribution ranges is similar to Hayami's view (1985), which identifies the relative populations (some distinct species?) with ***Volachlamys hirasei*** from Japan. However, such information on the distribution of ***V. hirasei*** should be reviewed hereafter, because as mentioned earlier, the Chinese species may be those that are distinct from the Japanese ones (the identical species have diverged by reproductive isolation).

In Japan, although ***Volachlamys hirasei*** once broadly inhabited the region, including southern Kanto and Atsumi areas, their inhabitancy has been restricted at present within the Seto Inland Sea and the Ariake Sea; only empty shells are collected from Mikawa Bay (Hayami, 1985). The author possesses several specimens of the two forms of ***V. hirasei*** from the Ariake Sea. The tone of their shell coloration is stronger than that of the specimens from the Seto Inland Sea, visually underscoring that they are somewhat different from each other. This is similar in photographs of the two forms of ***V. hirasei*** from the Ariake Sea illustrated by Sato (1975).

The Ariake Sea is considered to be a specialized region that has been semiclosed for a long time. It includes some peculiar animal species and an overall faunal affinity with the Asian continent (Sugano, 1981; Washio ***et al.***, 1996; Shimoyama, 2000). Genetic examinations of such marine animals inhabiting the Ariake Sea as the constricted tagelus, ***Sinonovacula constricta*** (Furukawa ***et al.***, 1996), the white flower croaker, ***Nibea albiflora*** (Menezes ***et al.***, 1990), the bluespotted mud hopper, ***Boleophthalmus pectinirostris*** (Furukawa ***et al.***, 1993) and the sea bass, ***Lateolabrax japonicus*** (Yokogawa ***et al.***, 1997), revealed that the Ariake populations have significantly diverged genetically from the other local populations. Those reports imply that the life forms in the Ariake Sea are unique and isolated from the other local populations. This may be consistent also with ***Volachlamys hirasei***.

As the genetic relationship between ***V. hirasei*** from the Ariake Sea and that from the Seto Inland Sea is very interesting, studies on the relationship should be performed hereafter. On the other hand, Kuroda (1932) described the number of radial costae of *V.* ***hirasei*** (yaminonishiki form) from Iyo (Ehime) to be significantly less. If that is true, it is necessary to obtain the specimens from the concerned waters and perform the morphological and genetic examinations.

Thus, the morphological, ecological and genetic examinations in the present study concluded that the two forms of ***Volachlamys hirasei*** are intraspecific morphological variations. However, such unknown biological information as behavioral ecology, propagative ecology and inheriting mechanism of the morphological characters still remains, thus requiring further studies. In malacological taxonomy, there are many similar cases to that of the two forms of ***V. hirasei***; they should be elucidated with the analytical methods in the present analytical studies.

Chapter 8 Phylogeny of shells in genus *Volachlamys* from Japan

Earlier chapters that examined the relationship between the two forms (awajichihiro and yaminonishiki) of ***Volachlamys hirasei*** by using morphological, ecological and genetic techniques, concluded that the two forms are intraspecific morphological variations, and proposed to unify the specific name.

Subsequently, this chapter examines the morphology and growth of fossil specimens of the genus ***Volachlamys***, collected from various Japanese formations, so as to carry out a phylogenetic study of the genus, elaborate further on their evolutional history and discuss taxonomic treatment.

Materials and methods

The fossil specimens examined in this chapter were collected from the Kazusa Formation, Nagasaki Prefecture (1.7 Ma: Otsuka and Furukawa, 1988; Otsuka ***et al.*** 1995), the Kitaarima Formation, Nagasaki Prefecture (0.9 Ma: Otsuka and Furukawa, 1988; Otsuka ***et al.*** 1995), the Maiko Formation, Hyogo Prefecture (the middle Pleistocene: Igawa and Ichihara), the Takatsukayama Formation, Hyogo Prefecture (0.41 Ma: Kato ***et al.*** 1999), the Atsumi Formation, Aichi Prefecture (Takamatsu silt facies, 0.44 Ma: Shimamoto ***et al.*** 1994), the Kioroshi Formation, inter-located between Chiba and Ibaraki Prefectures (0.125 Ma: Omori ***et al.*** 1986; Koike and Machida, 2001), and a coastal alluvium in Takamatsu, Kagawa Prefecture (0.006 Ma: Kawamura, 1988). The locales where the fossil specimens were collected are illustrated in Figure 18. In addition to those, the specimens of the living species, ***Volachlamys hirasei*** also were used for the study; specifically, 270 individuals from those specimens that were collected in May, 1989, and examined in Chapter 2. Data

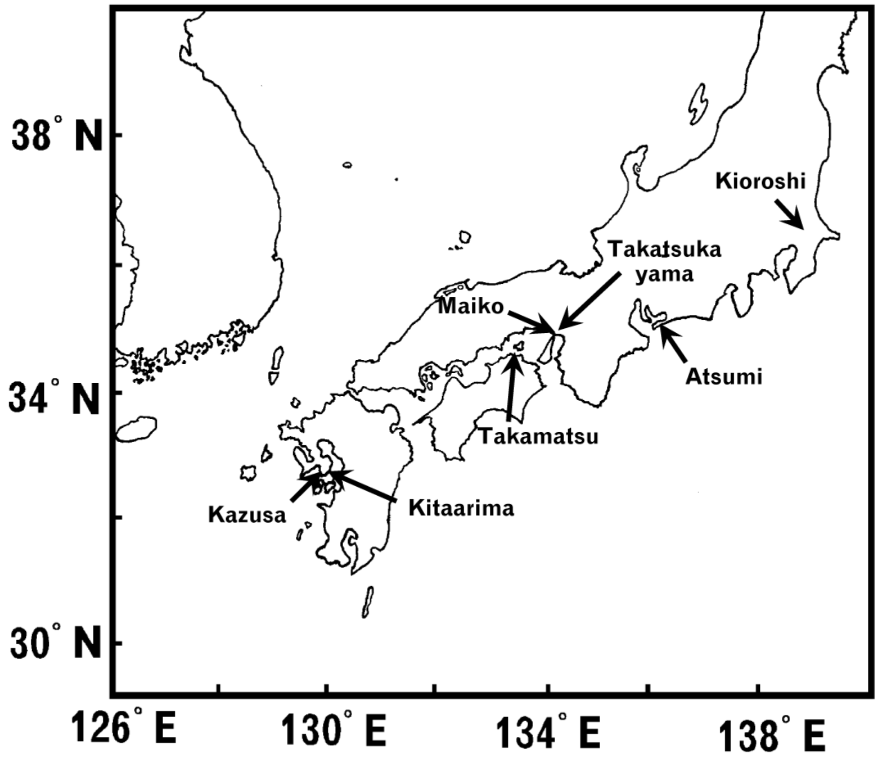

Fig. 18. Localities where fossil specimens were collected.

from the examined specimens are shown in Table 15; part of the specimens have been deposited in the Tokushima Prefectural Museum (TKPM), Osaka Museum of Natural History (OMNH) and the University Museum, the University of Tokyo (UMUT).

The specimen shells were measured morphologically, following the methods described in Chapter 2, to collect such data as shell length, shell height, shell width, auricle width, shell weight and the number of radial costae. In Chapter 2, although the shell width and shell weight were measured for the pair valves, this chapter measured those for single valves; it is very rare to obtain shell pairs in the case of fossil specimens. For the same reason, the radial costae number was counted for each single shell, even though the left valves only were measured for the shell pairs in Chapter 2. Regarding shell weight index (SWI), because the formula defined for SWI in Chapter 2 was for shell pairs, in an effort to standardize the shell weight, this chapter redefined that for the single shells, using the new formula as follows.

$$SWI = SW / (SL \times SH \times SWH) \times 10^5$$

(SW: shell weight [g]; SL: shell length [mm];
SH: shell height [mm]; SWH: shell width [mm])

For specimens from the living species, those items were remeasured for the single shells and SWI was recalculated.

Table 15. Data from specimens examined for phylogenetic examinations

Sample lot	Formation age (Ma)	Left valve			Right valve			Catalog No.
		N	Shell length		N	Shell length		
Type			Average	Range		Average	Range	
Kazusa	1.7							
Awajichihiro		30	38.0	14.6 − 51.3	35	38.8	19.0 − 53.3	TKPM-GFI3801
Kitaarima	0.9							
Awajichihiro		65	40.2	18.0 − 59.2	64	40.2	17.3 − 58.8	TKPM-GFI3802
Maiko	Middle Pleistocene							
Awajichihiro		46	56.2	23.0 − 70.8	40	47.5	18.3 − 71.7	OMNH-QM3870
Atsumi	0.44							
Awajichihiro		18	32.0	16.1 − 55.2	25	31.4	9.1 − 56.5	TKPM-GFI4493 (17 of 43)
Yaminonishiki					1	29.0	29.0 − 29.0	TKPM-GFI4494
Takatsukayama	0.41							
Awajichihiro		18	31.1	12.9 − 64.9	33	35.2	16.3 − 71.5	UMUT CM-16777
Kioroshi	0.125							
Awajichihiro		1	54.4	54.4 − 54.4				TKPM-GFI4269
Yaminonishiki					1	45.2	45.2 − 45.2	TKPM-GFI4586
Takamatsu	0.006							
Awajichihiro		11	29.0	17.1 − 39.2	11	25.8	16.7 − 41.9	
Yaminonishiki		7	34.1	23.0 − 41.9	10	23.2	12.3 − 34.5	
Living species								
Awajichihiro		221	43.8	30.5 − 59.2	221	43.6	30.4 − 59.9	
Yaminonishiki		49	45.8	33.9 − 53.1	49	45.4	30.2 − 52.6	

To compare the morphology of the specimens analytically, the principal component analysis (PCA) by the usual method (Arima and Ishimura, 1997) was introduced. For the analysis, specimens were deleted that lacked some of the measured items because of incomplete shells. Auricle width, radial costae number and SWI were adopted as variates for the analysis, while the shell length and shell width were deleted to avoid factor overlap since those factors were used to calculate SWI.

Because the annuli observed in the shell surface have been found to be yearly rings that are formed once a year (see Chapter 4), the annulus diameters were measured and the growth was analyzed with the methods described in Chapter 4. For the living species, the results of the growth analysis done on 929 awajichihiro and 258 yaminonishiki forms and described in Chapter 4, were used.

Results

Shell morphology

For the fossil specimens, samples from the Kazusa, Kitaarima, Maiko and Takatsukayama Formations comprised only the awajichihiro form, whereas those from the remaining formations included the yaminonishiki form. The yaminonishiki form appeared 1 of 44 individuals in the Atsumi sample, 1 of 2 individuals in the Kioroshi sample and 18 of 39 individuals in the Takamatsu sample (Table 15).

Table 16. Average values of shell characteristics in each sample lot

Sample lot ***Type***	Left valve					Right valve				
	Shell* height	Shell* width	Auricle* width	SWI	Costae number	Shell* height	Shell* width	Auricle* width	SWI	Costae number
Kazusa										
Awajichihiro	100.8	17.4	62.2	27.1	18.1	101.0	18.4	57.6	26.6	18.3
Kitaarima										
Awajichihiro	101.4	15.4	68.8	32.5	21.4	101.3	17.9	68.0	27.2	21.1
Maiko										
Awajichihiro	97.6	15.0	55.6	20.9	22.4	98.8	16.7	61.1	20.1	22.1
Atsumi										
Awajichihiro	101.3	15.0	75.6	32.6	17.1	102.9	16.4	79.5	26.8	16.8
Yaminonishiki						104.5	17.2	75.5	27.1	16.0
Takatsukayama										
Awajichihiro	101.1	14.9	80.9	28.9	18.2	100.1	16.3	79.1	28.5	18.8
Kioroshi										
Awajichihiro	98.0	15.8		27.1	17.0					
Yaminonishiki						92.3	14.2	75.2	24.0	
Takamatsu										
Awajichihiro	102.8	17.2	73.5	33.5	16.6	104.4	19.5	78.7	32.7	16.7
Yaminonishiki	105.9	16.9	71.9	34.3	15.5	106.8	18.0	77.3	28.5	15.1
Living species										
Awajichihiro	97.6	15.7	64.9	31.6	16.7	97.8	18.4	64.7	28.1	16.9
Yaminonishiki	99.1	14.4	63.3	30.7	15.8	99.7	17.2	63.8	27.8	15.1

* Percentage of shell length.

Table 16 summarizes average values of characters in the shells, and Figures 19 and 20 illustrate general aspects of typical individuals from each sample lot. Further, Figures 21–24 illustrate frequency distribution histograms for some characters.

As for shell height, many of the individuals from Kazusa, Kitaarima, Atsumi, Takatsukayama and Takamatsu samples have larger shell height than shell length (Fig. 21); the average values of (shell height)/(shell length) of those samples exceeded 100% (Table 16). Whereas, many of the individuals from the Maiko sample have smaller shell height than shell length (Fig. 21); the average values of (shell height)/(shell length) of the sample was below 100% (Table 16). Notably, an individual from the Maiko sample has very little angular expansion at the anterior and posterior margins of the shell; its shell shape is almost rounded (Fig. 19–C, Fig. 20–C). The individuals from the Takamatsu sample (both the awajichihiro and yaminonishiki forms) have particularly larger shell height than shell length. This is in contrast to the fact that the living species, which is very close both chronologically and geographically to the Takamatsu sample, tends to have smaller shell height than shell length (Fig. 21, Table 16).

The shell width of the right valves tended to be larger than that of the left valves in all the samples (Table 16), indicating greater swelling of the right valves in the

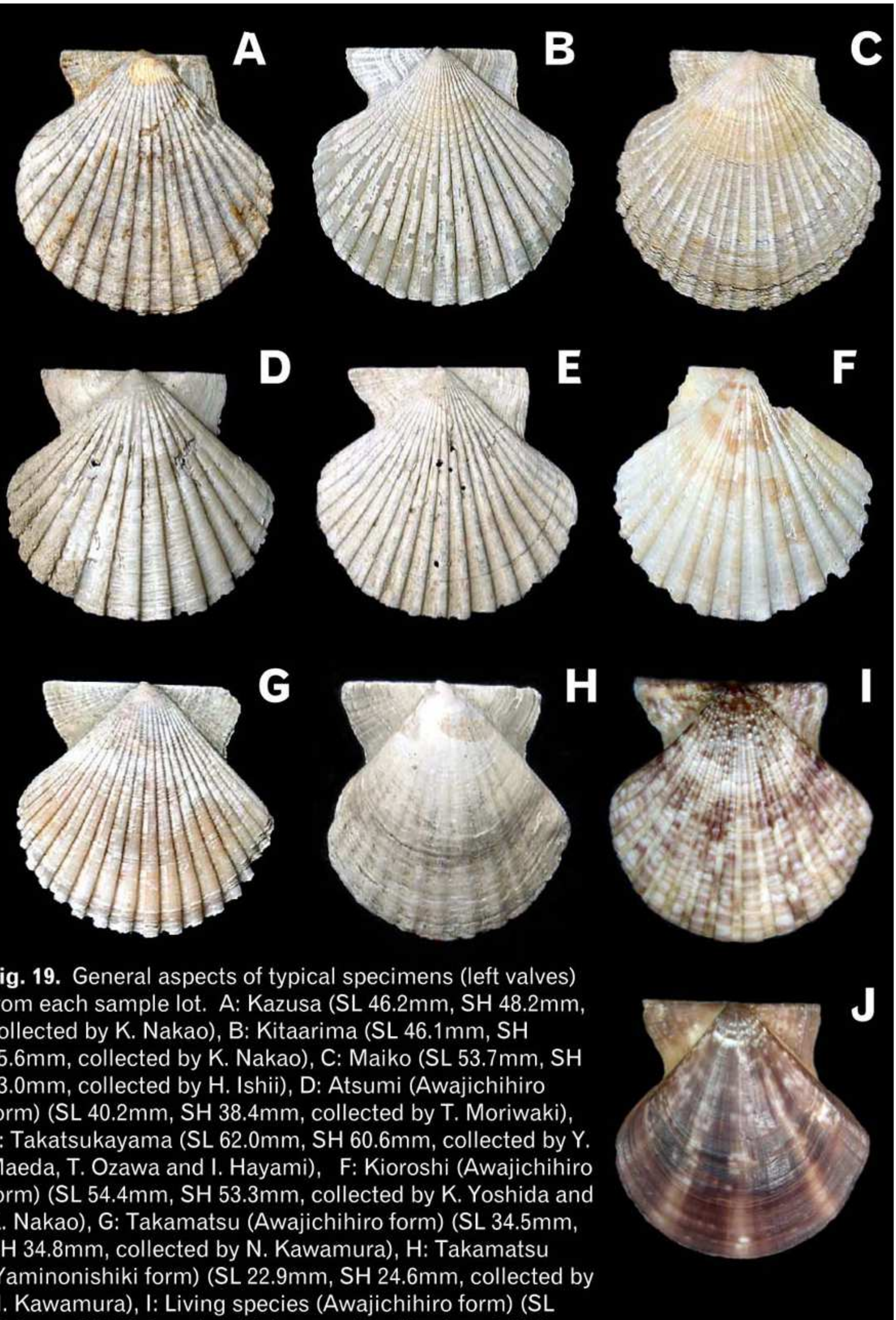

Fig. 19. General aspects of typical specimens (left valves) from each sample lot. A: Kazusa (SL 46.2mm, SH 48.2mm, collected by K. Nakao), B: Kitaarima (SL 46.1mm, SH 45.6mm, collected by K. Nakao), C: Maiko (SL 53.7mm, SH 53.0mm, collected by H. Ishii), D: Atsumi (Awajichihiro form) (SL 40.2mm, SH 38.4mm, collected by T. Moriwaki), E: Takatsukayama (SL 62.0mm, SH 60.6mm, collected by Y. Maeda, T. Ozawa and I. Hayami), F: Kioroshi (Awajichihiro form) (SL 54.4mm, SH 53.3mm, collected by K. Yoshida and K. Nakao), G: Takamatsu (Awajichihiro form) (SL 34.5mm, SH 34.8mm, collected by N. Kawamura), H: Takamatsu (Yaminonishiki form) (SL 22.9mm, SH 24.6mm, collected by N. Kawamura), I: Living species (Awajichihiro form) (SL 38.1mm, SH 37.8mm), J: Living species (Yaminonishiki form) (SL 37.7mm, SH 38.1mm).

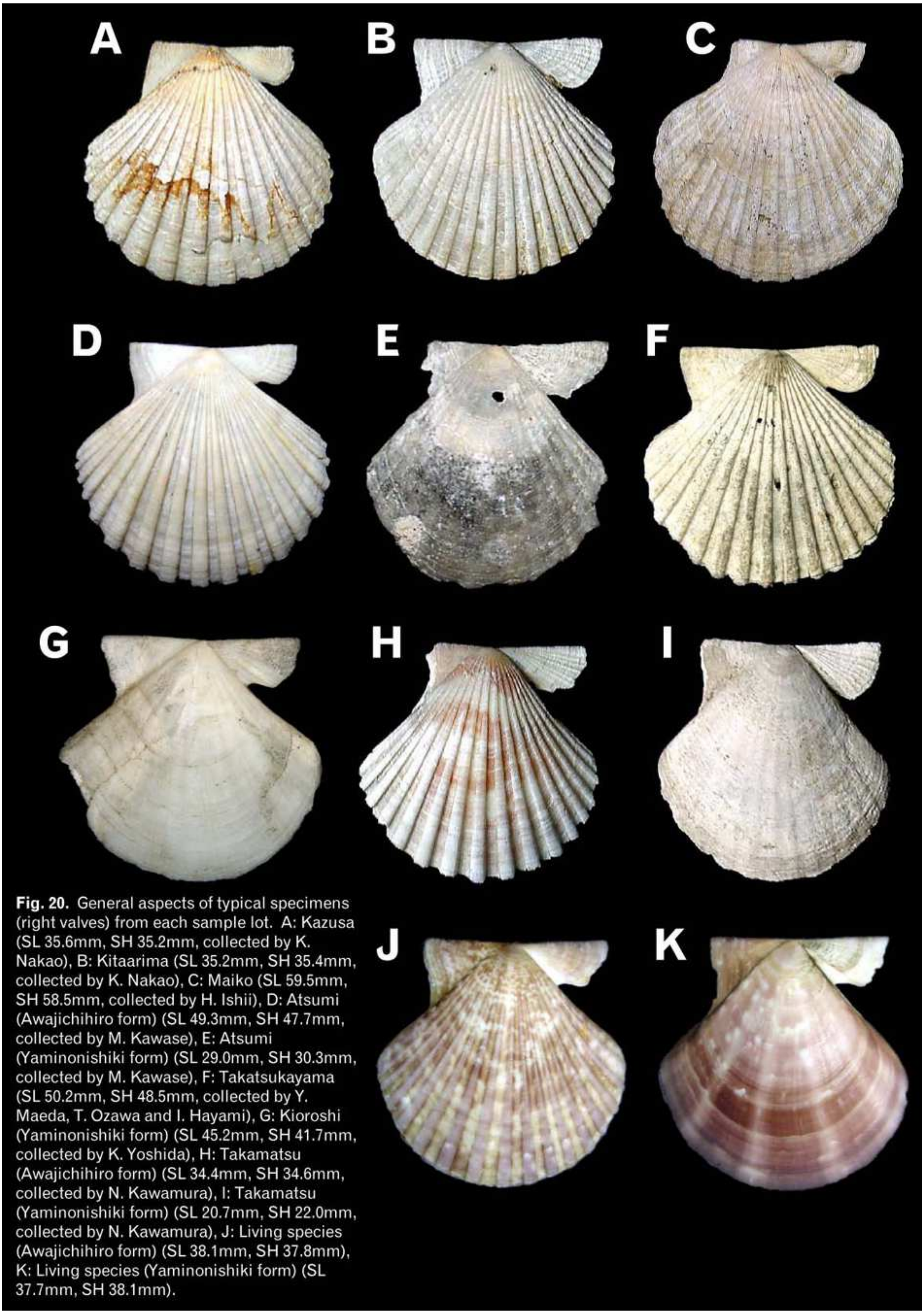

Fig. 20. General aspects of typical specimens (right valves) from each sample lot. A: Kazusa (SL 35.6mm, SH 35.2mm, collected by K. Nakao), B: Kitaarima (SL 35.2mm, SH 35.4mm, collected by K. Nakao), C: Maiko (SL 59.5mm, SH 58.5mm, collected by H. Ishii), D: Atsumi (Awajichihiro form) (SL 49.3mm, SH 47.7mm, collected by M. Kawase), E: Atsumi (Yaminonishiki form) (SL 29.0mm, SH 30.3mm, collected by M. Kawase), F: Takatsukayama (SL 50.2mm, SH 48.5mm, collected by Y. Maeda, T. Ozawa and I. Hayami), G: Kioroshi (Yaminonishiki form) (SL 45.2mm, SH 41.7mm, collected by K. Yoshida), H: Takamatsu (Awajichihiro form) (SL 34.4mm, SH 34.6mm, collected by N. Kawamura), I: Takamatsu (Yaminonishiki form) (SL 20.7mm, SH 22.0mm, collected by N. Kawamura), J: Living species (Awajichihiro form) (SL 38.1mm, SH 37.8mm), K: Living species (Yaminonishiki form) (SL 37.7mm, SH 38.1mm).

shells of this genus. The shell widths of both valves are significantly large in the Kazusa and Takamatsu samples (Table 16), showing a tendency of great swelling of the shells. Of the samples examined, the yaminonishiki form of the living species indicated the highest value in the difference between left and right valve shell widths, suggesting flatness of the left valves (Table 16).

The auricle width indicated unique tendencies by sample lots; in particular, the

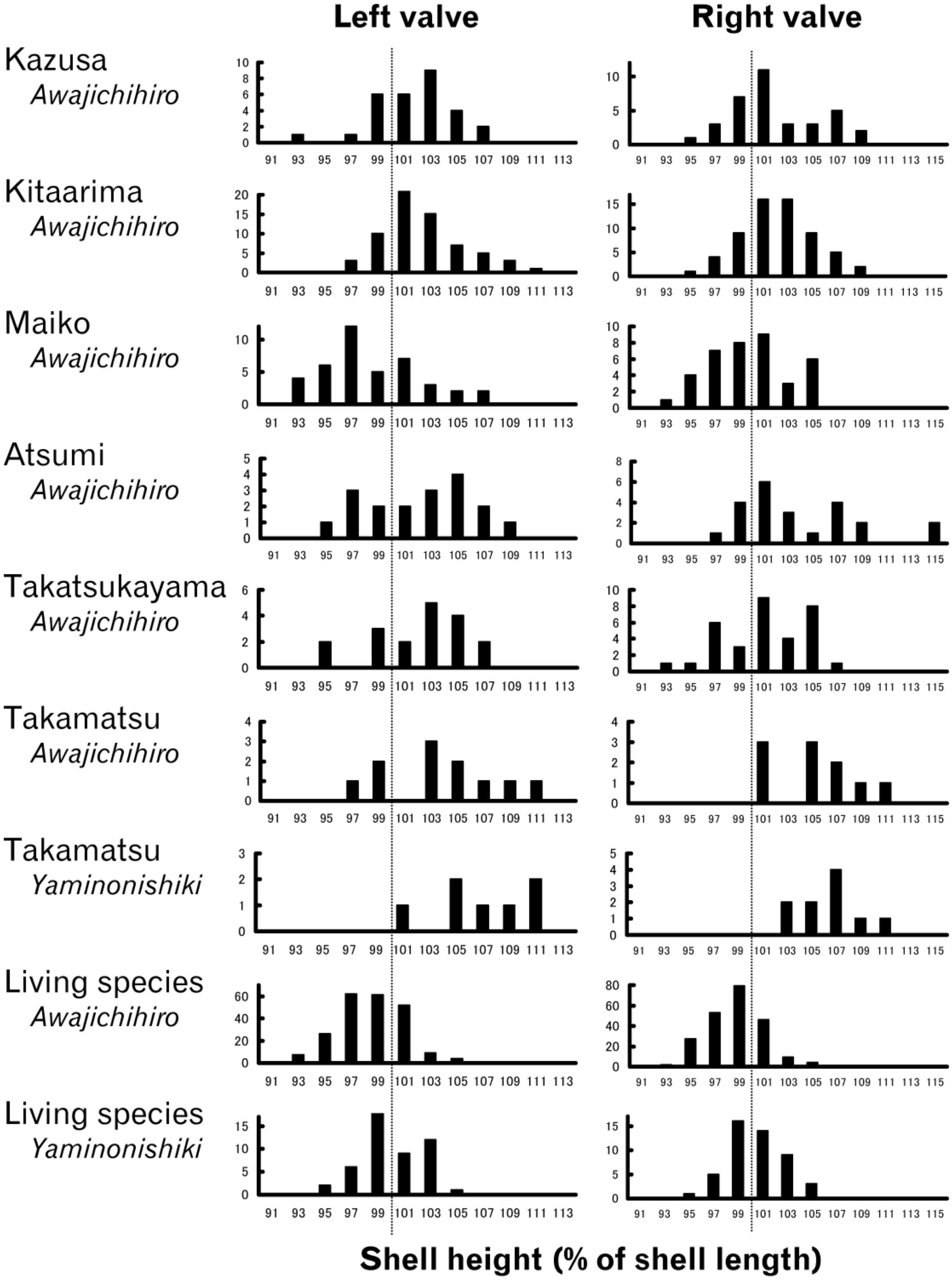

Fig. 21. Frequency distributions of shell height proportions of some sample lots. Longitudinal axes indicate numbers of individuals. Broken lines indicate markers of 100% (Shell height = Shell length).

average of (auricle width)/(shell length) of the Takatsukayama sample were extremely large values of around 80%, and the Atsumi and Takamatsu samples followed that, indicating about 75% (Table 16). In contrast, the auricle width of the Maiko sample was small; many of the individuals from the sample showed less than 60% (Fig. 22); the smaller auricle is recognized also in appearance (Fig. 19–C,

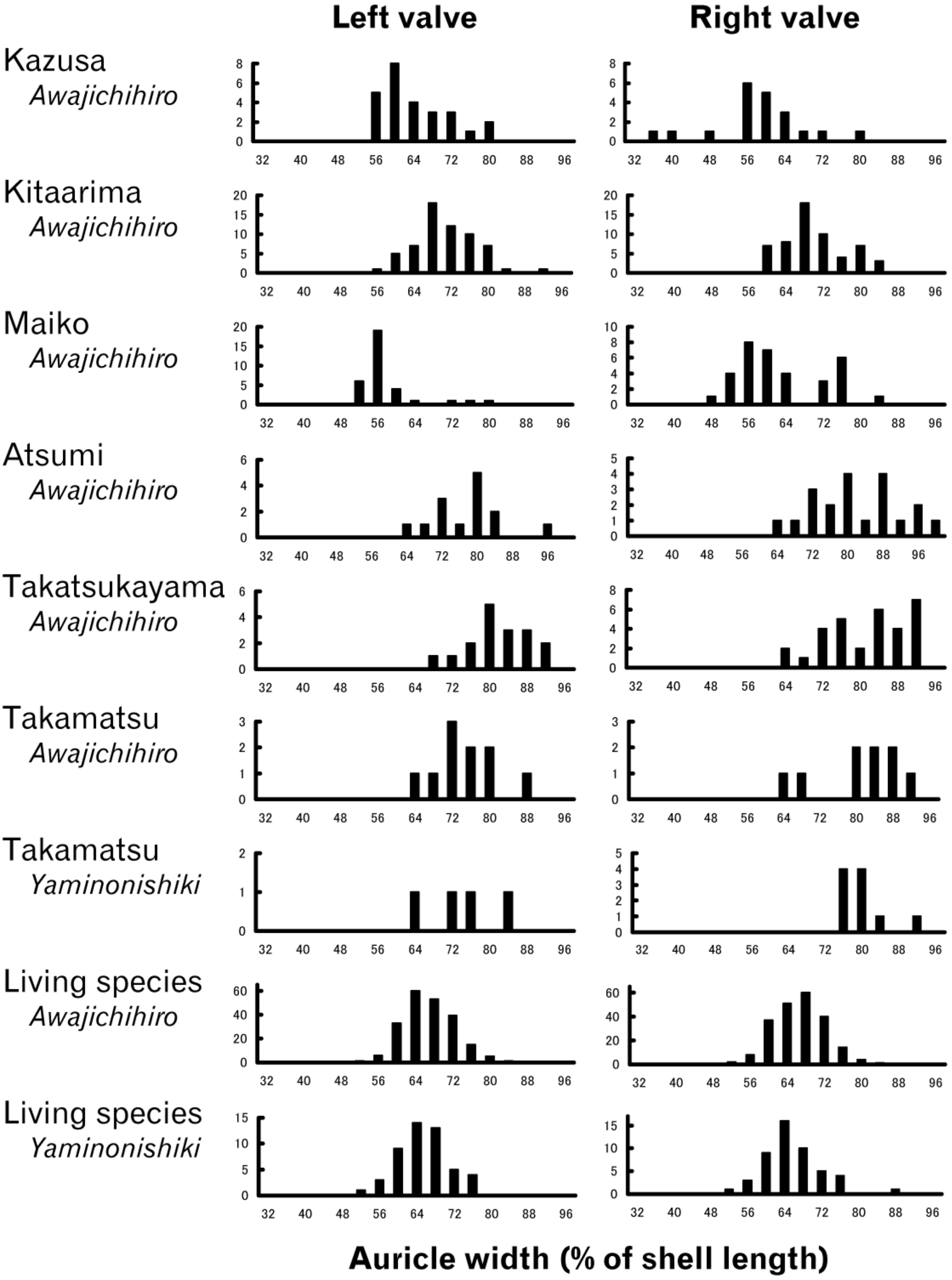

Fig. 22. Frequency distributions of auricle width proportions of some sample lots. Longitudinal axes indicate numbers of individuals.

Fig. 20−C). While the auricle width of the Kazusa, Kitaarima and living species samples indicated intermediate values between those (Table 16), it was remarkable that the auricle width of the Takamatsu sample considerably differed from that of the living species; nevertheless, they are very close to each other chronologically and geographically (Fig. 22, Table 16).

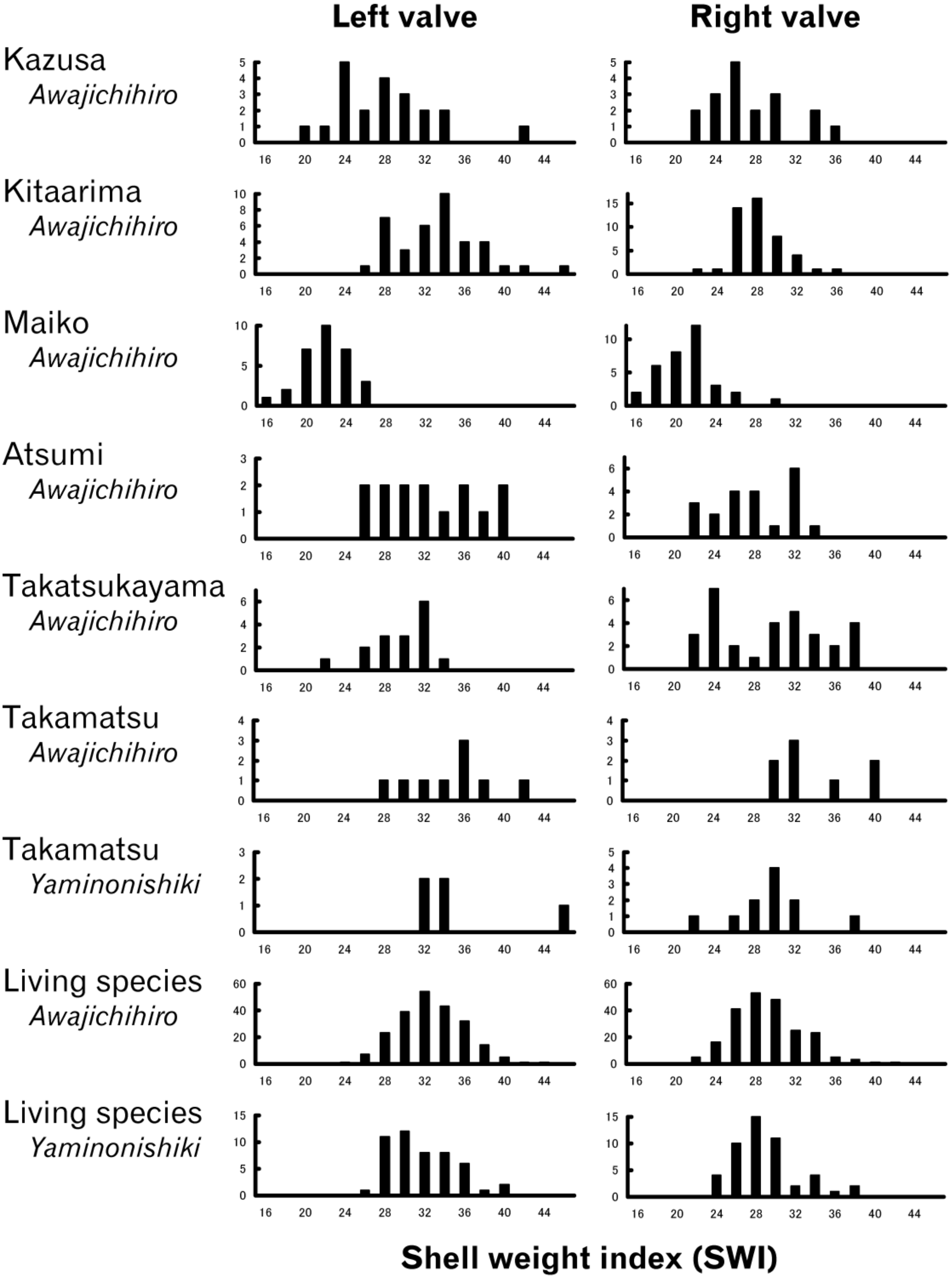

Fig. 23. Frequency distributions of shell weight index (SWI) of some sample lots. Longitudinal axes indicate numbers of individuals.

As for shell weight index (SWI), it was significant that that of the Maiko sample was considerably low (Fig. 23, Table 16). This suggests that the shells of the individuals from the Maiko sample are significantly thinner and lighter than those of the other individuals. Among the others, SWI values of the Kazusa and Takatsukayama samples tended to be lower than those of the remainders (Fig. 23, Table

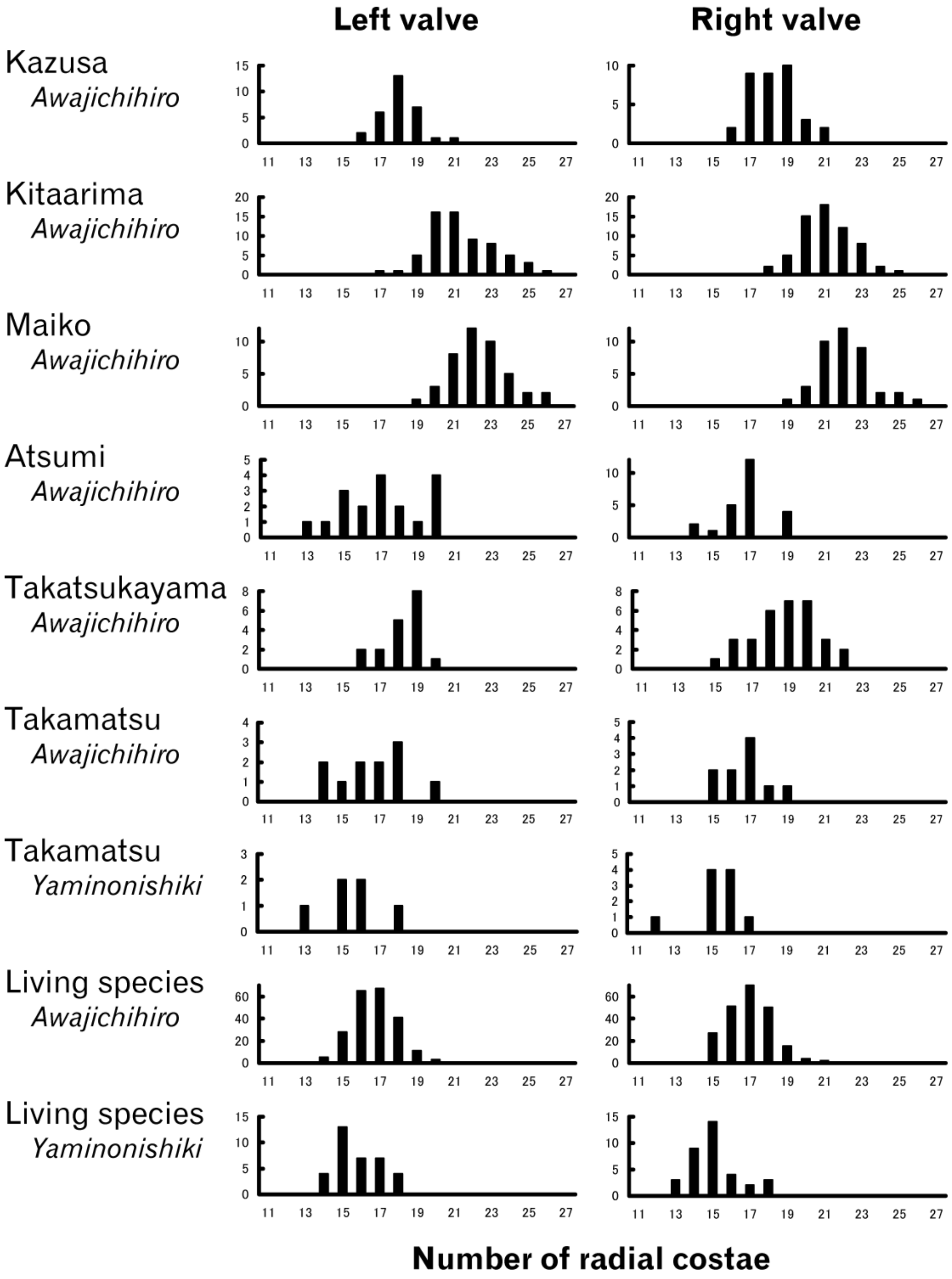

Fig. 24. Frequency distributions of radial costae numbers of some sample lots. Longitudinal axes indicate numbers of individuals.

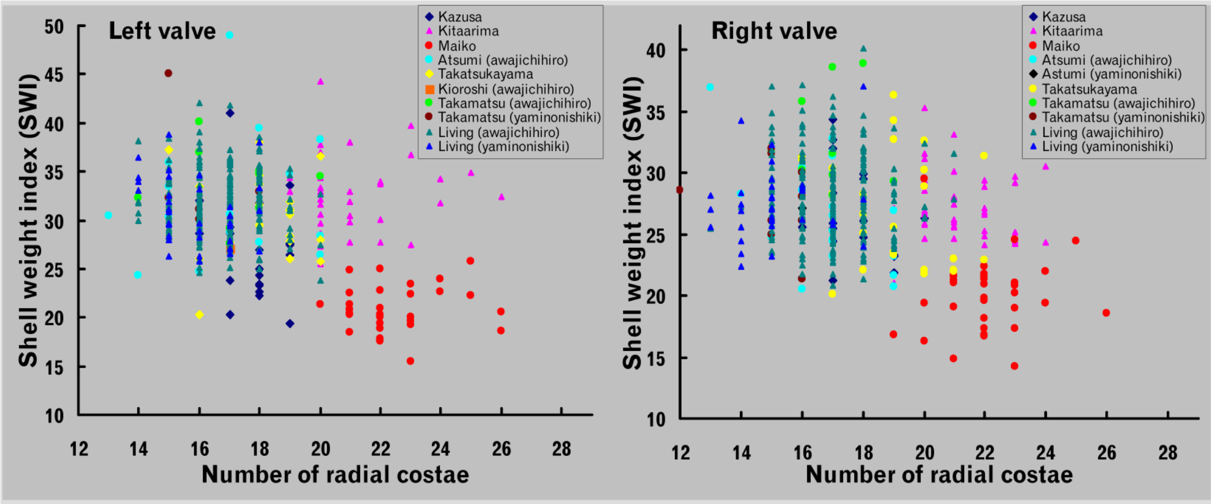

Fig. 25. Relationship between radial costae number and shell weight index (SWI).

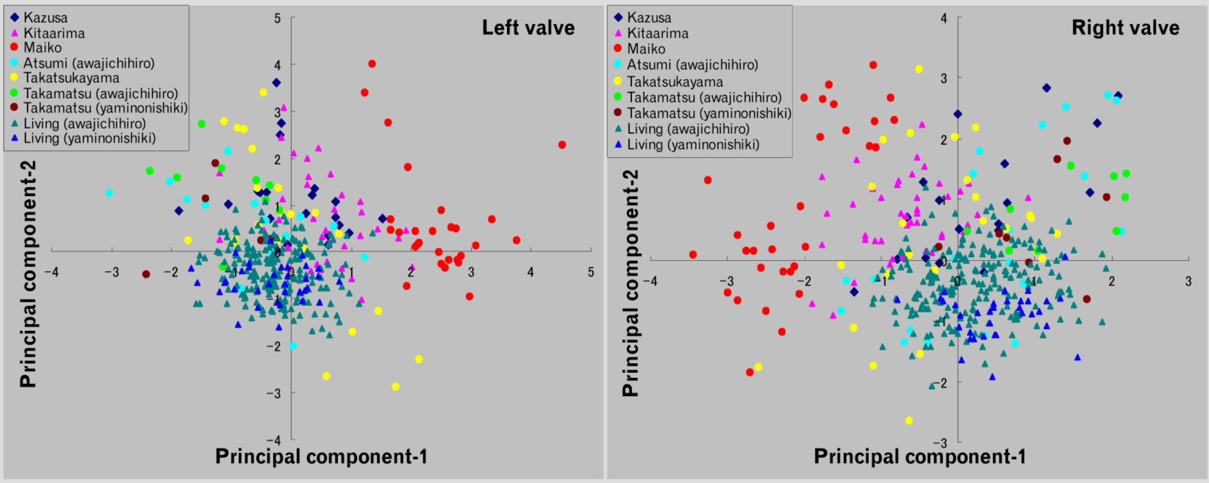

Fig. 26. Principal component analysis for the specimens examined.

16).

The number of radial costae was greatest in the Maiko sample; the average values were 22.4 and 22.1 in the left and right valves, respectively (Table 16), and the ranges were 17-26 and 18-25 in the left and right valves, respectively (Fig. 24). The Kazusa and Takatsukayama samples followed the Maiko sample; the average values were between 18 and 19 in both valves (Table 16). The average values of the other samples were between 16 and 17 in general, while those of the yaminonishiki forms from the Takamatsu and living species were lower than 16 (Table 16).

Relationship between the radial costae number and shell weight index (SWI) is illustrated in Fig. 25. When these two characters are combined, the individuals from the Maiko sample are separated from the others almost completely. Also, the individuals from the Kitaarima sample are well converged, though they are not separated completely from the others (Fig. 25).

Figure 26 shows results of principal component analysis (PCA) indicated with

Table 17. Average values of estimated shell lengths in each annulus group in each sample lot

Sample lot (***Type***)	r_1	r_2	r_3	r_4	r_5	r_6	r_7	r_8	r_9	r_{10}	r_{11}
Kazusa											
Awajichihiro	12.78	22.18	29.85	36.83	41.93	46.62	50.76				
Kitaarima											
Awajichihiro	11.14	20.25	28.66	34.89	40.09	44.75	49.76	52.86			
Maiko											
Awajichihiro	17.37	29.34	40.54	48.87	55.17	59.37	63.77				
Atsumi											
Awajichihiro	11.73	20.88	28.55	34.76	40.00	44.95	49.07	56.68			
Yaminonishiki	10.80	22.23	28.40								
Takatsukayama											
Awajichihiro	8.79	17.13	24.57	31.17	37.70	43.36	49.60	53.37	57.29	61.22	64.50
Kioroshi											
Awajichihiro	10.40	16.70	25.40	35.10	42.20	47.80	51.20				
Yaminonishiki	13.55	24.61	33.17	40.65							
Takamatsu											
Awajichihiro	12.35	21.50	29.53	36.70	39.89						
Yaminonishiki	11.76	20.56	26.88	31.32	37.27						
Living species											
Awajichihiro	16.53	25.13	32.12	37.46	41.61	45.01	48.08	51.46			
Yaminonishiki	15.85	24.46	32.00	37.80	42.27	45.73	48.43	51.60	48.15		

plots of individual principal component scores. Regarding these, the individuals from the Maiko sample were significantly separated from the others, while the individuals from the other samples were almost randomly distributed, although some samples showed a certain convergence by the individuals. This implies morphological peculiarity of the Maiko sample.

Age and growth

Initially, the Walford growth transformation formulae were conducted for the left and right valve groups in each sample, and differences between the parameters of the two formulae were examined with ***t*** test. From the results, no significant differences were recognized in all the samples; therefore, the growth analysis was performed with the pooled data of the left and right valves.

For each sample, the correlation formula of the shell height with shell length was calculated. Subsequently, the shell lengths at the annulus formation were estimated with the formulae, and average values of each annulus group were calculated (Table 17). Also, the parameters of the Walford growth transformation and von Bertalanffy growth formulae are summarized in Table 18. Further, Figure 27 shows growth curves of the samples by the von Bertalanffy growth formulae. Regarding those results, all the fossil samples except Maiko indicated rates of growth similar to those of the living awajichihiro and yaminonishiki forms; however, the Maiko sample clearly showed only excellent growth rate (Fig. 26, Table 17).

Table 18. Parameters of Walford growth transformation and von Bertalanffy growth formulae

Sample lot	Walford parameter[1]		von Bertalanffy praramter[2]		
Type	α	β	k	L_∞	t_0
Kazusa					
Awajichihiro	0.831	11.67	0.185	68.98	-0.106
Kitaarima					
Awajichihiro	0.844	11.07	0.170	70.78	-0.007
Maiko					
Awajichihiro	0.817	15.65	0.202	85.66	-0.123
Atsumi					
Awajichihiro	0.835	10.96	0.181	66.30	-0.084
Yaminonishiki	0.540	15.84	0.617	34.41	0.381
Takatsukayama					
Awajichihiro	0.895	9.27	0.111	87.90	0.055
Kioroshi					
Awajichihiro	0.905	9.82	0.100	102.90	-0.085
Yaminonishiki	0.816	13.42	0.204	72.77	-0.011
Takamatsu					
Awajichihiro	0.808	11.80	0.214	61.30	-0.053
Yaminonishiki	0.778	11.37	0.251	51.20	-0.039
Living species					
Awajichihiro	0.783	12.34	0.244	56.95	-0.404
Yaminonishiki	0.806	11.96	0.216	61.64	-0.378

[1] $L_{t+1} = \alpha L_t + \beta$ [2] $L_t = L_\infty (1 - e^{-k(t-t_0)})$

Discussion

The results in this chapter revealed that the Maiko sample is morphologically different from the other samples (Fig. 26) and that its growth rate is notably excellent (Fig. 27, Table 17). Of the morphological differences, it is significant that the shell weight index (SWI) of the Maiko sample is rather smaller than that of the other samples (Fig. 23, Table 16). Low SWI can indicate that shells of the Maiko individuals are thin; this may be related to the excellent growth rate. Chapter 4 examined growth of the living awajichihiro and yaminonishiki forms, and revealed that growth of the yaminonishiki form is better than that of the awajichihiro form. The reason for this phenomenon is thought to be that the yaminonishiki form can save calcium carbonate for shell creation; subsequently, the surplus calcium carbonate is used additionally for shell growth, consequently resulting in excellent growth. A very similar phenomenon is reported for two forms of the blistered scallop, ***Cryptopecten vesiculosus*** (strong and weak costa forms) (Hayami, 1984). The excellent growth of the Maiko sample also can be explained by such a hypothesis; the Maiko individuals can save the calcium carbonate for the shell creation because their shells are thin; subsequently, the surplus calcium carbonate is used additionally for shell growth, consequently resulting in excellent growth.

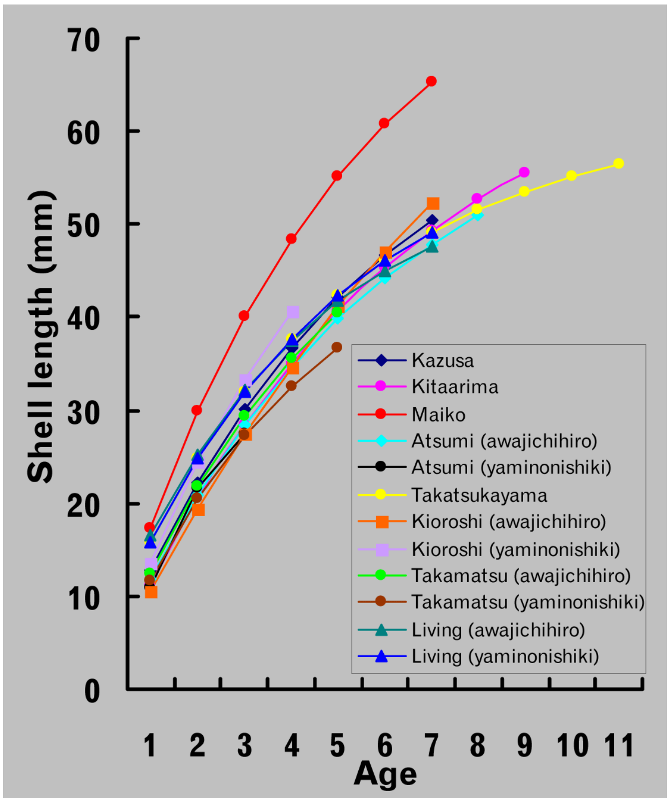

Fig. 27. Growth patterns of the sample lots by von Bertalanffy growth formulae.

Such biological characteristics of the Maiko sample obviously indicate that it is systematically a distinct population from the other samples. This may explain the other morphological characteristics of smaller auricle width and greater radial costae number in the Maiko sample. Because the Maiko individuals are almost completely separated from the others with respect to the radial costae number and SWI (Fig. 25), they should be treated as an independent species.

As for the samples other than the Maiko sample, although morphological characters including the radial costae number are unique to one another, samples with extremely specialized morphology were not recognized, including the living species (Figs. 21–24, Table 16). The growth patterns of those samples are similar to one another (Fig. 27, Table 17), inferring monophyly of the samples other than the Maiko sample. But even if those samples are monophyletic, considerable biological (ecological and genetic) differences can be expected among the samples, between which a time gap of hundreds of thousands of years exists. Therefore, on the concept of biological species (Wiley, 1991), the samples other than the Maiko sample might be independent species, respectively. However, given what presently is known about their morphological characteristics, there is no choice but to regard them as identical species because they cannot be completely distinguished. The individuals from the Takamatsu sample, which are very close to the living species chronologically and geographically, considerably differ morphologically from the living species (Figs. 21, 22, Table 16). This suggests the potential that morphological changes occurred within a very short span of geological time. This matter can support the treatment that the samples other than the Maiko sample are regarded to be a single species.

Concerning scientific names, the fossil specimens of the genus ***Volachlamys*** are generally supposed to be yaguranishiki (mukashichihiro), ***Volachlamys yagurai***, being regarded as the distinct species from the living ***V. hirasei*** (Hayami, 1985;

Fujiyama, 1986; Kaneko and Kajiyama, 1993). ***V. yagurai*** was newly described with the type specimen from the Maiko Formation (Makiyama, 1923). The present study revealed that the individuals from the Maiko Formation, the locale of ***V. yagurai***, possess sufficiently peculiar biological characteristics to be considered a distinct species from the living species, whereas such characteristics could not be detected from the fossil specimens from other formations. Although populations having weak costae (yaminonishiki form) may be regarded as ***V. hirasei***, it is difficult to identify whether a population is ***V. yagurai*** or ***V. hirasei*** when the entire specimen group consists of just a few individuals of the awajichihiro form. Individuals of the yaminonishiki form could potentially appear thereafter. Thus, the taxonomic treatment concluded that the individuals from the Maiko Formation are yaguranishiki, ***Volachlamys yagurai***, while for the other fossil specimens, the oldest specific name (Bavay, 1904) for the Japanese ***Volachlamys*** bivalves is adopted; that is, they are awajichihiro, ***Volachlamys hirasei***. The Japanese name of "awajichihiro" is appropriate because most of the fossil specimens are the awajichihiro form (Table 15).

Although the samples other than the Maiko sample are uniformly ***Volachlamys hirasei***, some of the samples, such as the Kitaarima sample, have somewhat peculiar morphological characteristics. Therefore, it may be reasonable to establish some subspecies for the fossil samples. This should be considered carefully with further examination of the other fossil specimens of the genus ***Volachlamys*** from the various formations, including newly discovered ones hereafter.

Tokunaga (1906) described a new fossil species of ***Pecten pulchellimus*** from Oji, Tokyo, which is quite similar to ***Volachlamys hirasei*** in appearance. Because the Tokyo Formation where ***P. pulchellimus*** was deposited is correlative with the Kioroshi Formation (Omori ***et al.*** 1986), ***P. pulchellimus*** is regarded to be the identical population with ***V. hirasei***. Consequently, ***P. pulchellimus*** is a junior synonym of ***V. hirasei***. Thereafter Yokoyama (1926) described a new fossil species of ***Pecten atsumiensis*** from the Atsumi Formation. Since photographs in the description were very similar to ***V. hirasei*** and the Atsumi Formation deposits ***V. hirasei***, ***P. atsumiensis*** also is a junior synonym of ***V. hirasei***.

Hayami (1985) considered the phylogeny of the Japanese ***Volachlamys*** bivalves. He hypothesized that the population from the Maiko Formation, which have many radial costae, chronologically transited into the population from the Takatsukayama and Kazusa Formations, which have somewhat fewer radial costae. Subsequently, the radial costae number further decreased chronologically, and they transited into the living population monophyletically as a consequence. However, a negative suggestion given for his hypothesis is that that the radial costae reduced chronologically because the radial costae number of the individuals from the Kazusa Formation (1.7 Ma) was fewer than that of the individuals from the Kitaarima Formation (0.9 Ma). Although he hypothesized that the Japanese ***Volachlamys*** bivalves are monophyletic, the present study revealed that at least two strains once existed around the Japanese waters.

Hashimoto and Maeda (1989) considered chronological order of the Takatsukayama and Maiko Formations based on the hypothesis of Hayami (1985). They proposed that the Takatsukayama Formation is younger because the radial costae number of so-called "***Volachlamys yagurai***" from the Takatsukayama Formation is fewer than those from the Maiko Formation. However, Hayami's hypothesis is invalidated by the present study. Moreover, comparison of the specimens from the Takatsukayama and Maiko Formations is not available because the specimens are distinct species from each other; consequently, Hashimoto and Maeda's opinion is baseless.

The present study determined that the fossil ***Volachlamys*** individuals (except those from the Maiko Formation) are monophyletic; among those, the weak costa form (yaminonishiki form) appeared 1 of 44 individuals in the Atsumi sample, 1 of 2 individuals in the Kioroshi sample and 18 of 39 individuals in the Takamatsu sample (Table 15). This suggests that the yaminonishiki form, which is an intraspecific morphological variation, appeared in the population in the middle Pleistocene. Thereafter, its frequency in the population increased chronologically; consequently, the population transited into the living population. The fact that fossils of the yaminonishiki form appear in the Kanto and Tokai regions in addition to the Seto Inland Sea and Ariake Sea, where the living populations dwell, infers that a common population inhabited a considerably broad area when the fossils lived, or there were environmental factors, such as elevation of sea level during the inter-glacial epochs, to expand the distribution easily. Thereafter, shallow and closed sea areas inhabitable for the population reduced; consequently, the living population is restrictively distributed at present.

Many of the problems concerning the genus ***Volachlamys*** have been resolved in the present study; however, there still are some problems to be examined, such as related ***Volachlamys*** species inhabiting China and the establishment of subspecies among the fossil samples. Hereafter, taxonomy of the genus ***Volachlamys***, including the exotic species, should be reviewed, and their phylogeny should be revealed.

Acknowledgments

The author heartily thanks Mr. Ken-ichi Nakao, Tokushima Prefectural Museum, for willingly providing many fossil shell specimens he personally collected, and much literature and information on geology. Also, I am very grateful to Drs. Nobuhiko Taniguchi and Shingo Seki, Faculty of Agriculture, Kochi University (present address of the former: Faculty of Agriculture, Tohoku University), who made available equipment for electrophoretic analyses and gave much support and advice.

The study of Chapter 8 could not have been carried out without the willing help of people noted below in obtaining the fossil shell specimens. Mr. Hisao Ishii, Osaka Museum of Natural History, loaned the Maiko sample, Dr. Itaru Hayami, ex-professor of Faculty of Science, Kanagawa University, loaned the Takatsukayama sample, Mr. Toru Moriwaki, Okazaki City, Aichi Prefecture, Mr. Motohiro Kawase, Faculty of Human Science, Aichi Mizuho University, and Mr. Kazuo Yano, Nagoya City, Aichi Prefecture, loaned the Atsumi sample, and Mr. Norihito Kawamura, Takamatsu High School, loaned the Takamatsu sample.

Dr. Hayami also made suggestions for the present analytic studies as a referee of the journal of malacology. I appreciate his insight and achievements as a pioneer of the study on ***Volachlamys hirasei***.

Dr. Yasuo Kondo, Faculty of Science, Kochi University, Dr. Akihiro Kijima, Field Science Center, Graduate School of Agriculture Science, Tohoku University, Dr. Yukio Hanamura, National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Miss Eriko Kita, ex-student of Faculty of Education, Kagawa University, Dr. Katsuhiko Wada, National Research Institute of Aquaculture (present address: National Research Institute of Fisheries Science, Fisheries Research Agency), Mr. Hiroshi Ieyama, Faculty of Education, Ehime University, and Mr. Kenji Mimoto, Kochi Prefectural Office, providing some literature.

Dr. Kondo also gave particular support and advice in creating the present thesis. Dr. Hanamura also provided available information on the pea crabs. Dr. Takashi Okutani, ex-professor of College of Bioresource Sciences, Nihon University, gave information and advice on Japanese names of ***Volachlamys hirasei***.

Finally, I wish to thank Elizabeth Paxton, ScratchCat, LLC, Alpharetta, Georgia, US, for checking the manuscript.

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