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Growth-related Conchological Changes in the Japanese True Limpets of the Family Nacellidae

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Abstract: Growth-related conchological changes in the basal shell length in 7 species and 1 subspecies of the Japanese nacellid limpets *Cellana nigrolineata*, *C. toreuma*, *C. grata grata*, *C. grata stearnsii*, *C. testudinaria*, *C. radiata*, *C. mazatlandica* and *C. enneagona* were examined. Shell measurements demonstrated frequent growth-related proportional changes; shell width, shell height and internal colored area width showed relative increases in many species and subspecies. In particular, relative increases were shown in most shell regions in *C. nigrolineata* and *C. mazatlandica*. In contrast, relative shell height decreased with growth in *C. enneagona*, and there were no growth-related proportional changes in *C. grata grata* or *C. radiata*. Although relative apex position shifted backward with growth in *C. mazatlandica*, it stayed stable in the other species and subspecies. Meristic characters of radial stripes in *C. nigrolineata* and radial costae in *C. grata stearnsii* and *C. mazatlandica* all showed apparent growth-related increases. Although color pattern compositions in the internal colored area varied by species and subspecies, significant growth-related changes were observed in *C. testudinaria* and *C. mazatlandica*, *i.e.*, new light color layers were gradually formed onto the dark brown basal layers observed in smaller individuals. In conclusion, many conchological characteristics of the Japanese nacellid limpets change with growth, and their patterns differ between species.

Keywords: Nacellidae, *Cellana*, Morphology, Growth-related change

Introduction

Conchological characteristics are important taxonomic criteria for shell-bearing mollusks. Although shell forms dramatically change with growth in a few taxa including the Strombidae and Siliquariidae, they are largely stable between variously sized shells within a single species in most other taxa (*e.g.*, Habe *et al*., 1994; Sasaki, 2010). Therefore, very few studies have ever examined growth-related conchological changes (*e.g.*, Vat, 2000; Cabral, 2007). However, if conchological characteristics, and particularly shell proportions, do gradually change with growth, this is important basic information for malacology. The true limpets of the family Nacellidae, which have a simple shell structure, are suitable materials for such a study. Thus, the present study examined basic conchological features of the Japanese nacellid limpets that change in proportion to growth in basal shell length.

Materials and Methods

Seven species and one subspecies from the family Nacellidae listed below occur in Japan, and all were examined in the present study. Their scientific names and identification follow Habe (1975), Habe & Kosuge (1967) and Sasaki (2000).

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Cellana nigrolineata (Reeve, 1839) (Japanese name: Matsuba-gai)
Cellana toreuma (Reeve, 1854) (Japanese name: Yome-ga-kasa)
Cellana grata grata (Gould, 1859) (Japanese name: Bekkō-gasa)
Cellana grata stearnsii (Pilsbry, 1891) (Japanese name: Ami-gasa)
Cellana testudinaria (Linnaeus, 1758) (Japanese name: Ō-bekkō-gasa)
Cellana radiata (Born, 1891) (Japanese name: Kuruma-gasa)
Cellana mazatlandica (Sowerby, 1839) (Japanese name: Kasa-gai)
Cellana enneagona (Reeve, 1854) (Japanese name: Shiwa-gasa)

Although there are some different taxonomic views regarding *Cellana grata grata* and *C. grata stearnsii*, the present study provisionally regarded them as subspecies following Habe (1975) and Habe & Kosuge (1967). Although both authors spelled the epithet "*stearnsi*," it was originally described by Pilsbry (1891) as *Patella* (*Helcioniscus*) *Stearnsii*. Thus, the present study corrected the epithet to "*stearnsii*" following the original spelling.

Some of specimens examined in the present study are deposited in the Natural History Museum and Institute, Chiba (CBM), the National Museum of Nature and Science (NSMT) and the Tokushima Prefectural Museum (TKPM). Material was also examined from the author's own collection (KY). Specific material property and collection localities are as follows.

Cellana nigrolineata (93 specimens): uncatalogued specimens (93) (KY), Aji, Kagawa Prefecture (Pref.). *Cellana toreuma* (45 specimens): uncatalogued specimens (KY), Yashima, Kagawa Pref. *Cellana grata grata* (45 specimens): uncatalogued specimens (45) (KY), Hiwasaura, Tokushima Pref. *Cellana grata stearnsii* (58 specimens): CBM-ZM 2662 (3), Hayama, Kanagawa Pref.; CBM-ZM 2683 (2), Mihama, Wakayama Pref.; CBM-ZM 163376 (2), Yoro I., Kagoshima Pref.; NSMT-Mo 1200 (3), Kagoshima Pref.; NSMT-Mo 39705 (3), 39706 (8), 41852, Shirahama, Wakayama Pref.; NSMT-Mo 41805, 77517 (2), 77518 (3), Okinoshima I., Kochi Pref.; NSMT-Mo 45872 (9), Cape Shionomisaki, Wakayama Pref.; NSMT-Mo 56942 (6), Minami-osumi, Kagoshima Pref.; NSMT-Mo 60583 (2), Tokara Is., Kagoshima Pref.; NSMT-Mo 77513, Suwanose I., Kagoshima Pref.; NSMT-Mo 77514, Wakayama Pref.; NSMT-Mo 77515 (8), 77516 (3), Amami I., Kagoshima Pref. *Cellana testudinaria* (34 specimens, KY): uncatalogued specimens (2), Amami I., Kagoshima Pref; uncatalogued specimen (1), Zamami I., Okinawa Pref; uncatalogued specimens (31), Iriomote I., Okinawa Pref. *Cellana radiata* (25 specimens): CBM-ZM 103587, Orchid I., Taiwan; CBM-ZM 163524, Dulan, Taiwan; CBM-ZM 148731, Singapore; CBM-ZM 163522, NSMT Mo-39732, 39733, 77519 (3), Minami-daito I., Okinawa Pref.; CBM-ZM 163523, Hateruma I., Okinawa Pref.; NSMT-Mo 39734, 41810 (2); TKPM-Mo 95 (4), Ishigaki I., Okinawa Pref.; uncatalogued specimen (personal collection of S. Yano), Yaku I., Kagoshima Pref.; uncatalogued specimens (3) (personal collections of S. Yano (1), Y. Hamamura (1) and KY (1), respectively), Amami I., Kagoshima Pref.; uncatalogued specimens (4) (KY), Miyako I., Okinawa Pref; *Cellana mazatlandica* (60 specimens): CBM-ZM 2681, 2694, 112997, 114264 (3), 128275 (4), NSMT-Mo 1198 (12), 1199 (10), 45813 (13), 61220 (6), uncatalogued specimens (9) (personal collections of S. Yano (1), Y. Hamamura (5) and KY (3), respectively), Ogasawara Is., Tokyo Metropolis. *Cellana enneagona* (143 specimens): CBM-ZM 2644 (18), Cebu I., Philippines; CBM-ZM 105309 (6 of 7), Kita-iwo I., Tokyo Metropolis; CBM-ZM 106067 (10 of 11), 106185 (2), 107382 (2), Pagan I., Northern Mariana Is.; CBM-ZM 106447 (1 of 2), 107360 (4), Anatahan I., Northern Mariana Is.; CBM-ZM 107373 (3), Guguan I., Northern Mariana Is.; CBM-ZM 163379, Philippines; NSMT-Mo 78477 (3), Osumi Pen., Kagoshima, Pref.; CBM-ZM 114267 (4), 151177, 128269 (3), 128276, NSMT-Mo 1206 (5), 1233 (11), 4197, 4413 (2), 77520 (4), 77521 (7), 77522 (2), 77523 (7), uncatalogued specimens (5) (personal collections of Y. Hamamura (3) and KY (2), respectively), Ogasawara Is., Tokyo Metropolis; NSMT-Mo 45870 (12), 45871, 77537 (27), Hachijo I., Tokyo Metropolis.

Some measurements defined in Fig. 1 were taken using calipers (minimum scale 0.1 mm);

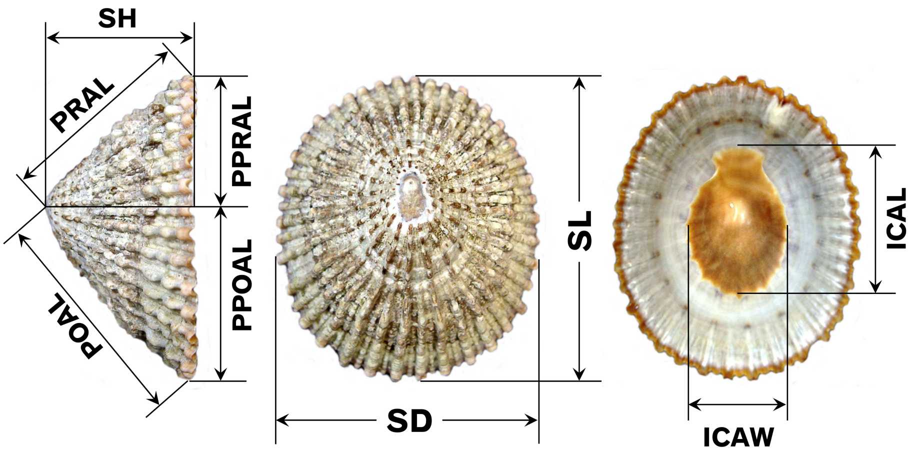

Fig. 1. Definition and terminology of measured regions in the shell. Abbreviations: SL, shell length; SWH, shell width; SH, shell height; PRAL, pre-apex length; POAL, post-apex length; PPRAL, projective pre-apex length; PPOAL, projective post-apex length; ICAL, internal colored area length; ICAW, internal colored area width.

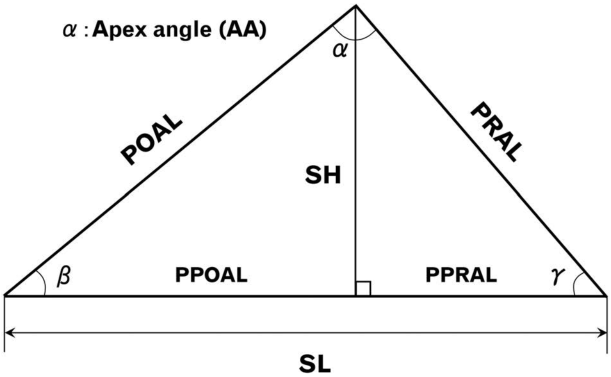

Fig. 2. A triangle model applied to a lateral section of the shell. Abbreviations are referred to Fig. 1.

shells were also weighed with an electric balance (minimum scale 0.01 g). Although the internal colored area (ICA) of a few *C. toreuma* individuals was colorless (almost transparent), its outline could be recognized as a distinct layer, allowing measurement of its length (ICAL) and width (ICAW).

The application of a triangle model to a lateral section of the shell (Fig. 2) allows estimation of the apex angle (α) and the other two angles (β and γ) using trigonometric functions. By the law of cosines, the following formulae are created:

$\cos\alpha = (\mathrm{PRAL}^2 + \mathrm{POAL}^2 - \mathrm{SL}^2)/(2 \times \mathrm{PRAL} \times \mathrm{POAL})$
$\cos\beta = (\mathrm{POAL}^2 + \mathrm{SL}^2 - \mathrm{PRAL}^2)/(2 \times \mathrm{POAL} \times \mathrm{SL})$
$\cos\gamma = (\mathrm{PRAL}^2 + \mathrm{SL}^2 - \mathrm{PROL}^2)/(2 \times \mathrm{PRAL} \times \mathrm{SL})$
where SL, shell length; PRAL, pre-apex length; POAL, post-apex length.

Subsequently, the apex angle (α, degrees) was found with the inverse trigonometric function (arccos α), further the projective pre-apex length (PPRAL) and projective post-apex length (PPOAL) were calculated also by the law of cosines as follows.

$$PPOAL = POAL \times \cos \beta$$
$$PPRAL = PRAL \times \cos \gamma$$

Because some species have meristic characters, *i.e*., radial stripes in *C. nigrolineata* and radial costae in *C. grata stearnsii* and *C. mazatlandica*, the stripes and costae in a shell were counted as whole units.

Measured shell portion dimensions (PD) were expressed as proportions of SL (PD/SL). In addition, proportions of PRAL/POAL, PPRAL/PPOAL and ICAW/ICAL were calculated, and relationships between SL and these proportions were graphically shown. Among these, the latter three proportions were tested for growth-related changes, *i.e*., the proportions (P) were made regress with SL ($P = a$ SL $+ b$), and the *t* test to examine significance of its slope for a null hypothesis ($a = 0$) was performed following Zar (2010). When a is significantly different from 0, the proportion is regarded as change with growth. This procedure was applied also for the apex angle (AA).

For evaluation of the other proportions, it would be inappropriate to apply the above method because their regressions include the same variate of SL on both sides of the formulae ($SL = a$ PD/SL $+ b$). Therefore, it was determined whether PD changes with growth isometrically or allometrically, *i.e*., regressions between SL and PD were transformed into natural logarithms (ln) ($\ln PD = a \ln SL + b$), and the *t* test to examine significance of its slope for a null hypothesis ($a = 1$) was performed, also following Zar (2010). When a is significantly different from 1, PD is regarded as changing allometrically, *i.e*., its proportion increases or reduces with growth. This procedure was applied also to those meristic characters in which growth-related increases were recognized, in order to examine whether the increases are isometric or allometric.

In the statistical inferences mentioned above, because multiple tests are applied simultaneously to all species or subspecies, multiple comparisons were introduced for the results of the *t* test under a null hypothesis of isometric growth in any shell regions. For such comparisons, risk percentages for the *t* values were corrected according to total test counts using the Holm-Bonferroni method (Holm, 1979).

The regression between SL and the shell weight (SW) was calculated for its logarithm-transformed formula ($\ln SW = a \ln SL + b$) excluding individuals whose shells were significantly chipped. Subsequently, the *t* test to examine the significance of the relative growth coefficient value (a) for a null hypothesis ($a = 3$), which supports stable growth, were performed also following Zar (2010).

Cellana nigrolineata exhibits radially striped and dappled patterns, with a complex of the two, and these forms have been shown to be an intraspecific phenotypic variation (Nakano *et al*., 2010; Yokogawa, 2014). The examples of this species examined in the present study were primarily of the radial striped form, but one specimen each of the dappled and complex forms were included in the conchological analyses. The radial stripes were counted in the complex form but were uncountable in the dappled form.

Colors in the ICA were observed and classified into patterns (Fig. 3) (including colorless in *C. toreuma*). When the ICA was showed two colors, and the proportion of the less dominant color was within *ca*. 10%, it was classified as the more dominant color. When the less dominant color area was over 10%, however, it was regarded as a complex of both colors. The ICA could not be observed in some specimens, including uncatalogued specimen (1 of 45) from *C. toreuma*, NSMT-Mo 41810 (1 of 2) from *C. radiata* and CBM-ZM 114267 (3 of 4) from *C. enneagona*, because they contained dried soft parts.

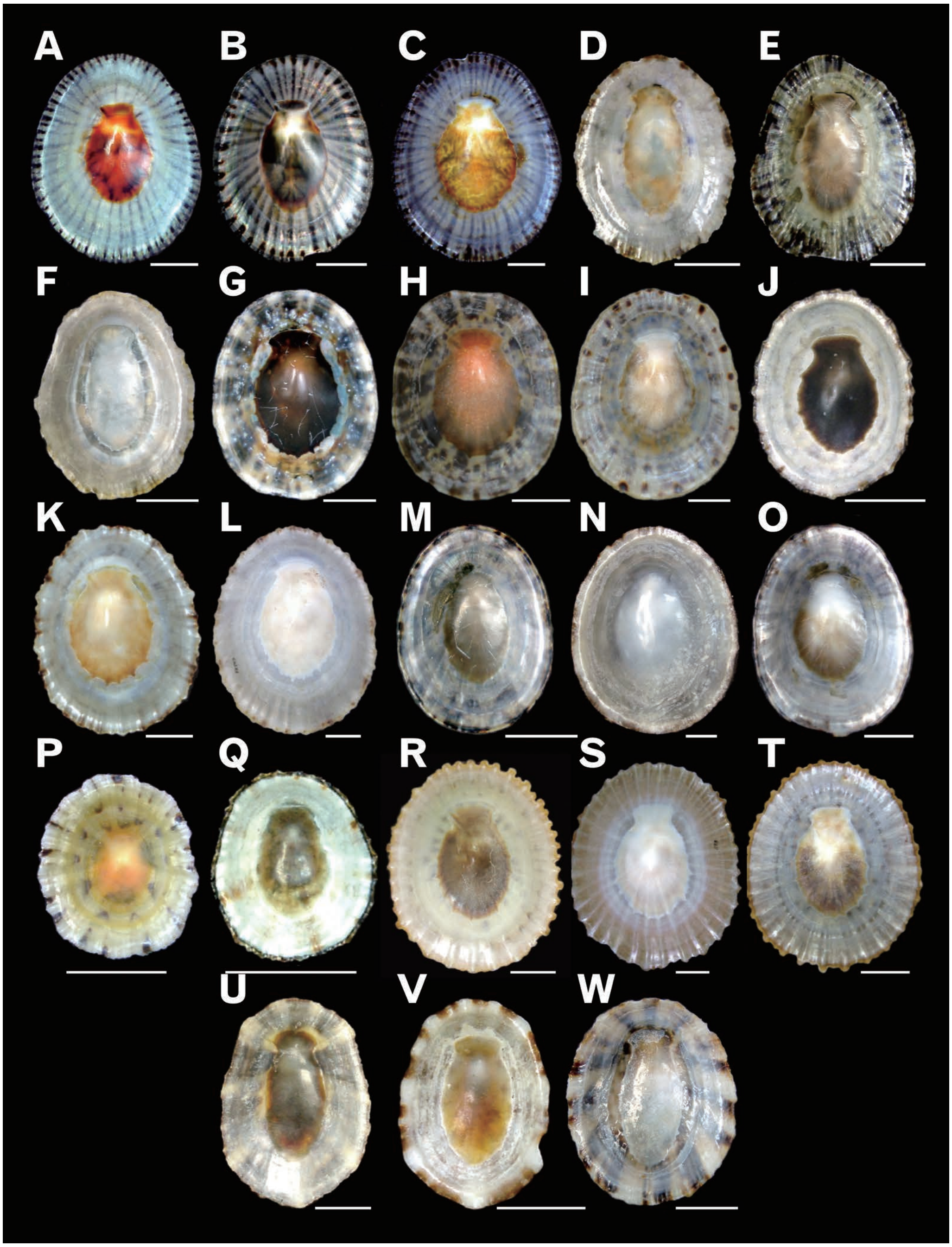

Fig. 3. Typical color patterns of internal colored areas in Japanese nacellid limpets. **A–C**. *Cellana nigrolineata* (A, dark red; B, dark brown; C, light brown). **D–F**. *C. toreuma* (D, light brown; E, dark brown; F, white). **G–I**. *C. grata grata* (G, dark brown; H, dark orange; I, light brown). **J–L**. *C. grata stearnsii* (J, dark brown; K, light brown; L, white). **M–O**. *C. testudinaria* (M, dark brown; N, pale; O, dark brown/pale complex). **P**, **Q**. *C. radiata* (P, orange; Q, dark brown). **R–T**. *C. mazatlandica* (R, dark brown; S, cream; T, dark brown/cream complex). **U–W**. *C. enneagona* (U, dark brown; V, brown; W, white). Scale bars = 10 mm.

Results

Growth-related changes of shell proportions

Over all species and subspecies, the *t* tests to examine allometric or isometric growth showed significance for a number of the shell regions (Table 1), and those to examine correlations between shell length (SL) and other shell proportions indicated that some of the latter changed significantly with growth (Table 2). In particular, significance was recognized in most shell regions in *Cellana nigrolineata* and *C. mazatlandica*, but by contrast significance was recognized only in the internal colored area dimensions in *C. toreuma* and *C. testudinaria* and no significance was recognized in any shell regions in *C. grata grata* or *C. radiata* (Tables 1, 2).

Shell width (SWH) as a proportion of length (SWH/SL) increased with growth in *C. nigrolineata*, *C. mazatlandica* and *C. enneagona*, whereas it did not change in the other species and subspecies (Fig. 4; Table 1). In the former two species, it was apparent that the shells were expanding posteriorly with growth (Figs. 5, 6). Shell height (SH) increased relative to SL with growth in *C. nigrolineata*, *C. mazatlandica* and *C. grata stearnsii*, whereas it did not change in the other species and subspecies (Fig. 7, Table 1). In the former two species, it is very clear that SH increases with growth (Figs. 5, 6). The proportions of pre-apex length (PRAL) and post-apex length (POAL) were closely related to SH, significantly increasing with growth in the species and subspecies in which SH/SL increased (Table 1). Also, the apex angle (AA) was related to SH, significantly decreasing with growth (negative slope values) in the species and subspecies in which SH/SL increases (Tables 1, 2), indicating that AA becomes more acute according to apex elevation. On the other hand, it was notable that SH/SL significantly decreased with growth in *C. enneagona* and accordingly its AA increased with growth (Fig. 7H; Table 2).

Although the PPRAL/PPOAL ([projective pre-apex length]/[projective post-apex length]) ratio, which indicates relative apex position, did not change in most species and subspecies, it significantly increased with growth in *C. mazatlandica* (from *ca*. 60% to *ca*. 80%) (Fig. 8G), showing that the apex position moves relatively backward with growth. Photographs of a size series of *C. mazatlandica* demonstrate that the apex position shifts with growth (Fig. 6). Among the 6 species and 1 subspecies with a stable apex position, it was notable that *C. radiata* shows considerably high PPRAL/PPOAL values (*ca*. 70%) (Fig. 8F), indicating that its apex was located near to the center of the shell, unlike the other species and subspecies. In *C. mazatlandica*, the PPRAL/PPOAL values also reflected the same level as *C. radiata* in the larger individuals (Fig. 8G).

Growth-related changes of meristic characters

Counts of the radial stripes (RS) in *C. nigrolineata* and those of the radial costae (RC) in *C. grata stearnsii* and *C. mazatlandica* clearly increased with growth (Fig. 9). Photographs of size series of *C. nigrolineata* and *C. mazatlandica* demonstrate that RS and RC clearly increase with growth (Figs. 5, 6). The *t* tests to examine whether such growth-related increases are allometric or isometric showed high significance of negative allometry for all characters (Table 1), indicating that the direct relationships between SL and these meristic characters were expressed with power regressions as follows.

C. nigrolineata	$RS = 4.369 \times SL^{0.640}$	($r = 0.761$)
C. grata stearnsii	$RC = 6.792 \times SL^{0.514}$	($r = 0.797$)
C. mazatlandica	$RC = 11.885 \times SL^{0.356}$	($r = 0.844$)

Relationships between shell length and weight

The relationship between shell length (SL) and weight (SW) ($\ln SW = a \ln SL + b$) showed

Table 1. Regression parameters (slope and intercept) and correlation between logarithm-transformed shell portion and meristic counts, together with results of *t* tests to examine significance of slopes for a null hypothesis (slope = 1) for Japanese nacellid limpets (abbreviations are referred to Fig. 1).

Regression	Slope	Intercept	*r*	*t*
Cellana nigrolineata (*n* = 93)				
ln SL–ln SWH	1.065	–0.488	0.988	3.692**
ln SL–ln SH	1.472	–3.120	0.953	9.336***
ln SL–ln PRAL	1.205	–1.624	0.965	5.792***
ln SL–ln POAL	1.066	–0.556	0.989	3.807**
ln SL–ln ICAL	1.113	–1.005	0.974	4.101***
ln SL–ln ICAW	1.111	–1.416	0.955	3.003*
ln SL–ln RS[1]	0.640	1.475	0.761	–6.074***
Cellana toreuma (*n* = 45)				
ln SL–ln SWH	1.043	–0.420	0.990	1.847
ln SL–ln SH	1.110	–2.244	0.910	1.432
ln SL–ln PRAL	1.001	–1.067	0.942	0.021
ln SL–ln POAL	1.001	–0.316	0.988	0.041
ln SL–ln ICAL	1.194	–1.220	0.971	4.293***
ln SL–ln ICAW	1.322	–2.250	0.968	6.063***
Cellana grata grata (*n* = 45)				
ln SL–ln SWH	0.997	–0.218	0.988	–0.130
ln SL–ln SH	1.263	–1.965	0.885	2.599
ln SL–ln PRAL	1.107	–1.097	0.888	1.222
ln SL–ln POAL	1.059	–0.470	0.980	0.360
ln SL–ln ICAL	0.979	–0.404	0.960	–0.485
ln SL–ln ICAW	1.039	–1.006	0.925	0.595
Cellana grata stearnsii (*n* = 58)				
ln SL–ln SWH	1.029	–0.338	0.996	2.306
ln SL–ln SH	1.217	–1.712	0.928	3.337*
ln SL–ln PRAL	1.157	–1.198	0.971	4.146**
ln SL–ln POAL	1.081	–0.532	0.989	3.823**
ln SL–ln ICAL	0.989	–0.460	0.977	–0.372
ln SL–ln ICAW	0.997	–0.879	0.961	–0.088
ln SL–ln RC[2]	0.514	1.916	0.797	–9.249***
Cellana testudinaria (*n* = 34)				
ln SL–ln SWH	1.045	–0.410	0.993	2.059
ln SL–ln SH	1.136	–1.857	0.937	1.824
ln SL–ln PRAL	1.005	–0.908	0.955	0.090
ln SL–ln POAL	1.020	–0.333	0.995	1.093
ln SL–ln ICAL	1.043	–0.699	0.987	1.452
ln SL–ln ICAW	1.165	–1.598	0.986	4.733***
Cellana radiata (*n* = 25)				
ln SL–ln SWH	1.005	–0.181	0.978	0.120
ln SL–ln SH	0.996	–1.132	0.815	–0.025
ln SL–ln PRAL	0.995	–0.567	0.936	–0.066
ln SL–ln POAL	0.984	–0.330	0.971	–0.314
ln SL–ln ICAL	1.114	–0.885	0.956	1.571
ln SL–ln ICAW	1.262	–1.681	0.922	2.311
Cellana mazatlandica (*n* = 60)				
ln SL–ln SWH	1.038	–0.326	0.998	4.341***
ln SL–ln SH	1.240	–1.835	0.968	5.669***
ln SL–ln PRAL	1.170	–1.182	0.985	6.245***
ln SL–ln POAL	1.046	–0.475	0.992	2.621*
ln SL–ln ICAL	1.053	–0.799	0.984	2.104
ln SL–ln ICAW	1.081	–1.286	0.982	2.981*
ln SL–ln RC[2]	0.356	2.475	0.844	–21.751***
Cellana enneagona (*n* = 143)				
ln SL–ln SWH	1.036	–0.420	0.990	2.895*
ln SL–ln SH	0.910	–1.280	0.881	–2.683*
ln SL–ln PRAL	0.950	–0.830	0.945	–1.797
ln SL–ln POAL	0.981	–0.219	0.989	–1.567
ln SL–ln ICAL	0.956	–0.331	0.966	–2.006
ln SL–ln ICAW	1.120	–1.536	0.942	3.521**

[1] RS: Radial stripe counts [2] RC: Radial costae counts

Asterisks indicate significance of *t* vales; single, double and triple asterisks indicate 5%, 1% and 0.1% levels after Holm-Bonferroni correction by species (together with items in Table 2), respectively.

Table 2. Regression parameters (slope and intercept) and correlation between shell length (SL) and shell proportions plus apex angle, together with results of *t* tests to examine significance of slopes for a null hypothesis (slope = 0) for Japanese nacellid limpets (abbreviations are referred to Figs. 1 and 2).

Regression	Slope	Intercept	*r*	*t*
Cellana nigrolineata (*n* = 93)				
SL–PRAL/POAL	0.204	49.171	0.382	3.834**
SL–PPRAL/PPOAL	0.057	45.006	0.100	0.930
SL–ICAW/ICAL	0.003	65.751	0.006	0.060
SL–AA	–0.442	136.338	–0.608	–7.098***
Cellana toreuma (*n* = 45)				
SL–PRAL/POAL	0.006	47.367	0.008	0.054
SL–PPRAL/PPOAL	–0.014	41.903	–0.018	–0.120
SL–ICAW/ICAL	0.269	47.576	0.325	2.228
SL–AA	–0.184	139.443	–0.127	–0.837
Cellana grata grata (*n* = 45)				
SL–PRAL/POAL	0.086	60.713	0.064	0.422
SL–PPRAL/PPOAL	–0.051	50.330	–0.035	–0.232
SL–ICAW/ICAL	0.083	64.792	0.091	0.598
SL–AA	–0.360	115.906	–0.307	–2.117
Cellana grata stearnsii (*n* = 58)				
SL–PRAL/POAL	0.114	63.479	0.225	1.725
SL–PPRAL/PPOAL	–0.034	51.476	–0.053	–0.399
SL–ICAW/ICAL	0.007	67.416	0.020	0.146
SL–AA	–0.314	109.008	–0.521	–4.572***
Cellana testudinaria (*n* = 34)				
SL–PRAL/POAL	–0.040	55.184	–0.091	–0.514
SL–PPRAL/PPOAL	–0.052	42.726	–0.125	–0.712
SL–ICAW/ICAL	0.199	55.623	0.482	3.108*
SL–AA	–0.024	113.078	–0.047	–0.266
Cellana radiata (*n* = 25)				
SL–PRAL/POAL	0.057	80.524	0.040	0.191
SL–PPRAL/PPOAL	0.068	71.928	0.037	0.176
SL–ICAW/ICAL	0.514	59.898	0.347	1.736
SL–AA	0.049	105.955	0.028	0.135
Cellana mazatlandica (*n* = 60)				
SL–PRAL/POAL	0.219	68.452	0.674	6.942***
SL–PPRAL/PPOAL	0.243	54.646	0.568	5.260***
SL–ICAW/ICAL	0.043	66.332	0.245	1.922
SL–AA	–0.267	110.627	–0.562	–5.179***
Cellana enneagona (*n* = 143)				
SL–PRAL/POAL	–0.070	51.418	–0.093	–1.103
SL–PPRAL/PPOAL	0.014	39.656	0.017	0.199
SL–ICAW/ICAL	0.285	43.617	0.448	5.879***
SL–AA	0.240	115.105	0.280	3.469**

Asterisks indicate significance of *t* vales; single, double and triple asterisks indicate 5%, 1% and 0.1% levels after Holm-Bonferroni correction by species (together with items in Table 1), respectively.

highly significant correlations in all species and subspecies (Table 3). Among these, the resulting *a* values (relative growth coefficients) significantly exceeded 3 in *C. nigrolineata*, *C. testudinaria* and *C. mazatlandica*, supporting the relative increase in SW with growth. In particular, *C. nigrolineata* had the *a* value of 3.350, which showed a high significant difference from 3 (Table 3), demonstrating a high degree of relative SW increase with growth. On the other hand, because the *a* values of the other species and subspecies showed no significant difference from 3 (Table 3), their SL–SW regressions were regarded as stable relationships. The direct relationships between SL and SW were expressed with power regressions as follows:

C. nigrolineata $\mathrm{SW} = 1.176 \times 10^{-5} \times \mathrm{SL}^{3.350}$ ($r = 0.984$)
C. toreuma $\mathrm{SW} = 1.979 \times 10^{-5} \times \mathrm{SL}^{3.089}$ ($r = 0.989$)
C. grata grata $\mathrm{SW} = 7.369 \times 10^{-5} \times \mathrm{SL}^{2.935}$ ($r = 0.938$)

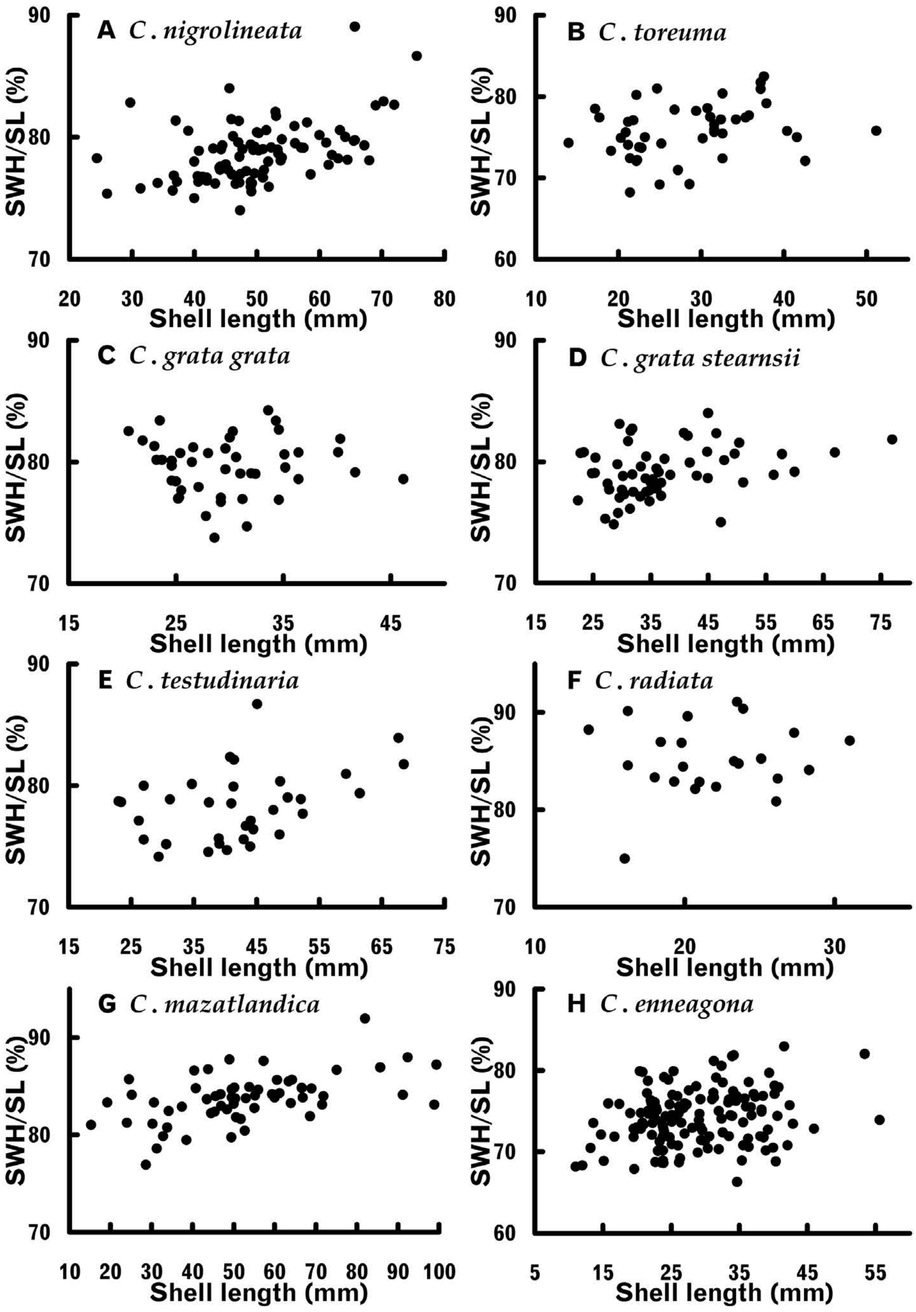

Fig. 4. Relationships between shell length and SWH/SL ([Shell width]/[Shell length]) proportions in Japanese nacellid limpets.

C. grata stearnsii $SW = 5.929 \times 10^{-5} \times SL^{3.090}$ ($r = 0.961$)
C. testudinaria $SW = 2.803 \times 10^{-5} \times SL^{3.187}$ ($r = 0.987$)
C. radiata $SW = 6.233 \times 10^{-5} \times SL^{3.037}$ ($r = 0.963$)
C. mazatlandica $SW = 3.948 \times 10^{-5} \times SL^{3.140}$ ($r = 0.991$)
C. enneagona $SW = 3.791 \times 10^{-5} \times SL^{2.961}$ ($r = 0.971$)

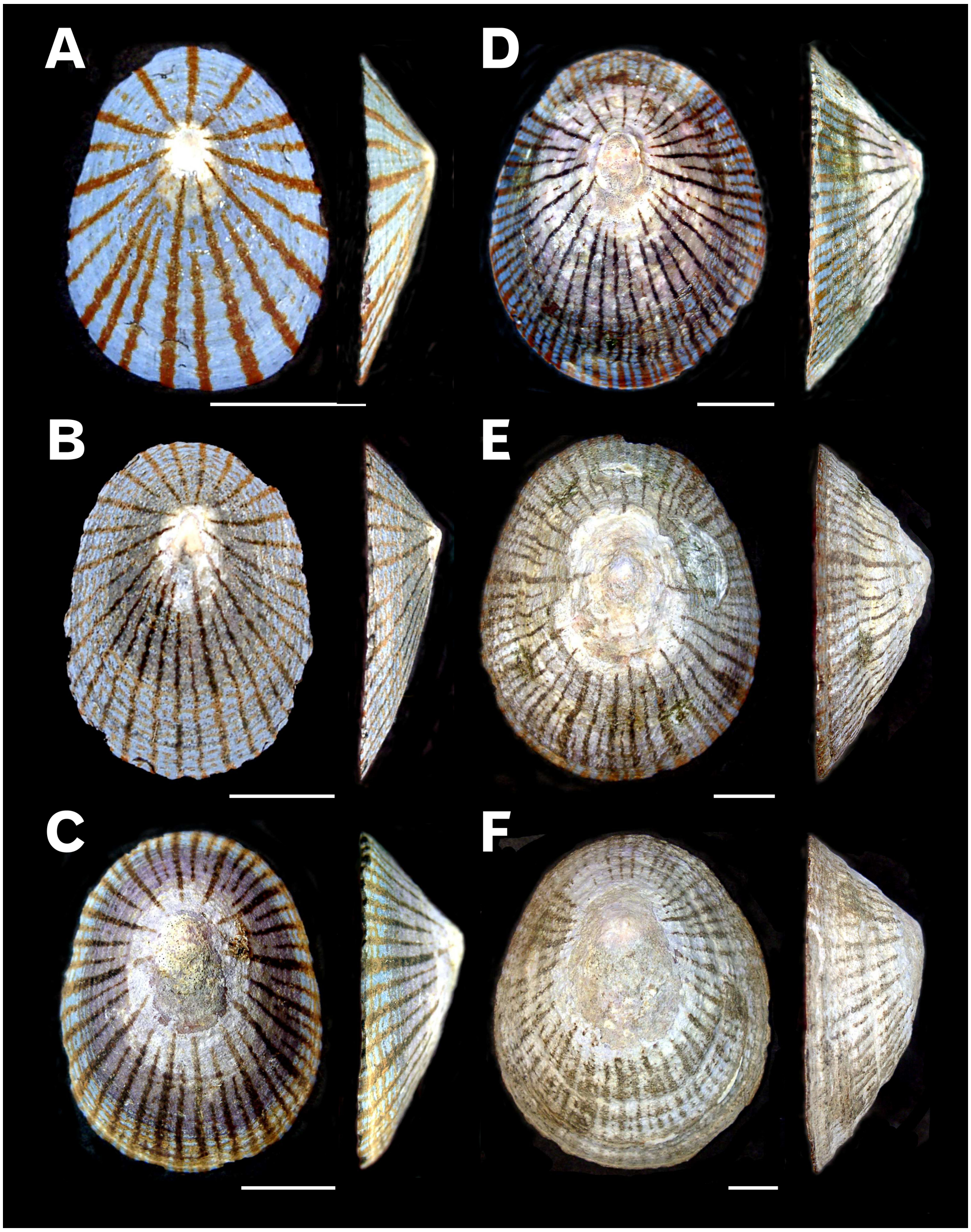

Fig. 5. Apical and lateral aspects of *Cellana nigrolineata* arranged in order of increasing size. SL 22.0 mm (**A**), SL 32.5 mm (**B**), SL 36.5 mm (**C**), SL 44.5 mm (**D**), SL 56.0 mm (**E**), SL 69.0 mm (**F**). Scale bars = 10 mm.

Growth-related changes of internal colored area

The length (ICAL) and width (ICAW) of the internal colored area (ICA) were measured. The ICAL/SL ratio significantly increased with growth in *C. nigrolineata* and *C. toreuma*, but did not change in the other species and subspecies (Fig. 10; Table 1). The ICAW/SL ratio significantly increased with growth (having positive allometry) in all species except *C. grata grata*, *C. grata stearnsii* and *C. radiata* (Table 1). Further, the ICAW/ICAL ratio significantly increased with

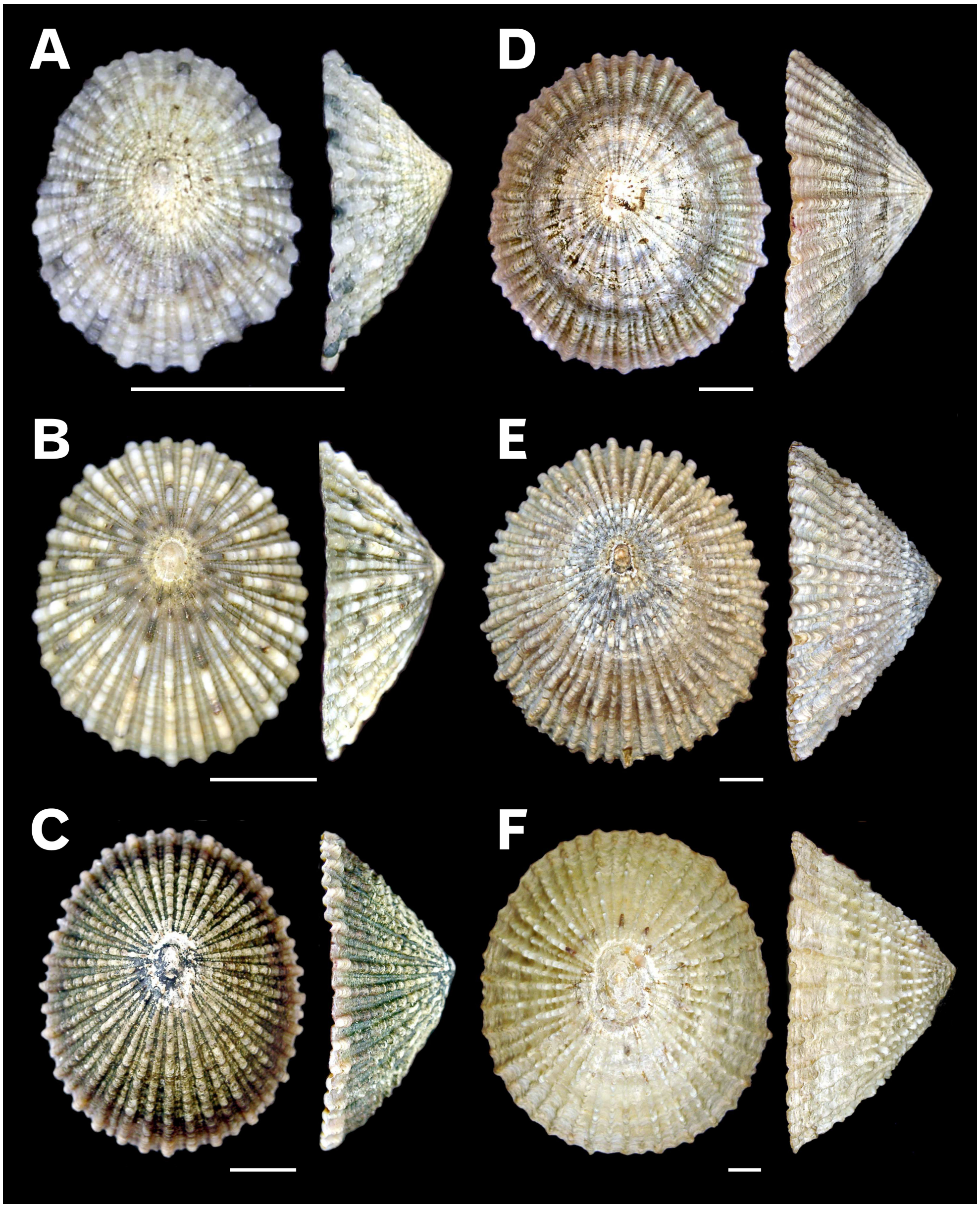

Fig. 6. Apical and lateral aspects of *Cellana mazatlandica* arranged in order of increasing size. SL 15.3 mm (**A**), SL 30.6 mm (**B**), SL 49.4 mm (**C**), SL 60.6 mm (**D**), SL 75.1 mm (**E**), SL 99.4 mm (**F**). Scale bars = 10 mm.

growth in *C. testudinaria* and *C. enneagona*, unlike in the other species and subspecies (Table 2).

Regarding the ICA color patterns, each species and subspecies had a distinctive variation (Fig. 3; Table 4). Among these, *C. testudinaria* and *C. mazatlandica* showed an apparent color transition with growth. In the former, the ICA of all the smaller individuals (SL ≤ 30 mm) was dark brown (Fig. 3M). Dark brown/pale complex and pale patterns (Fig. 3O) gradually increased in the middle size class (30 < SL ≤ 50 mm), and the ICA was all pale (Fig. 3N) in the larger

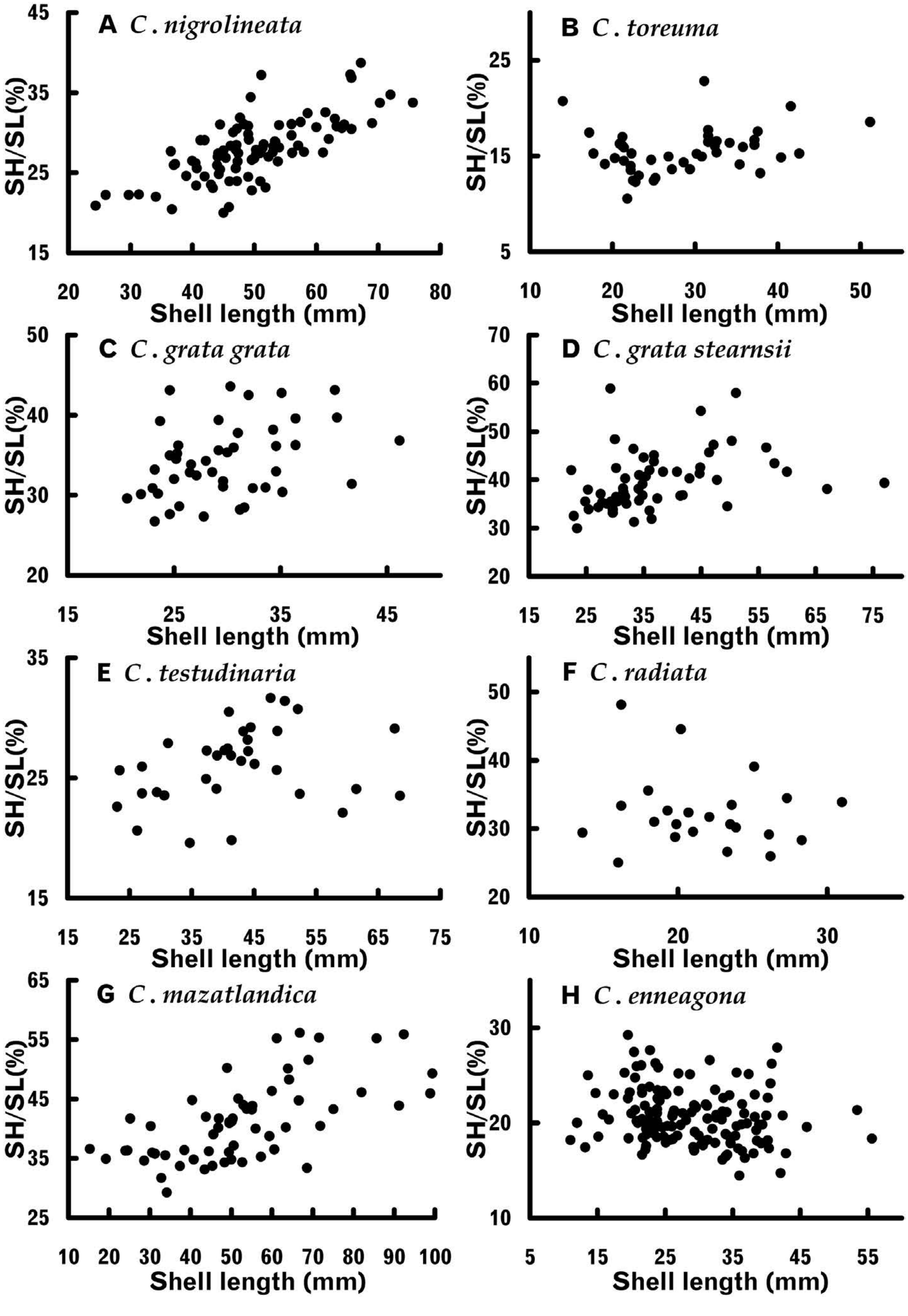

Fig. 7. Relationships between shell length and SH/SL ([Shell height]/[Shell length]) proportions in Japanese nacellid limpets.

individuals (SL > 50 mm) (Table 4). In the latter, the ICA of all the smaller individuals (SL ≤ 30 mm) was also dark brown (Fig. 3R). Dark brown/cream complex and cream patterns (Fig. 3T) gradually increased in the middle size class (30 < SL ≤ 70 mm), and the ICA was all cream (Fig. 3S) in the larger individuals (SL > 70 mm) (Table 4).

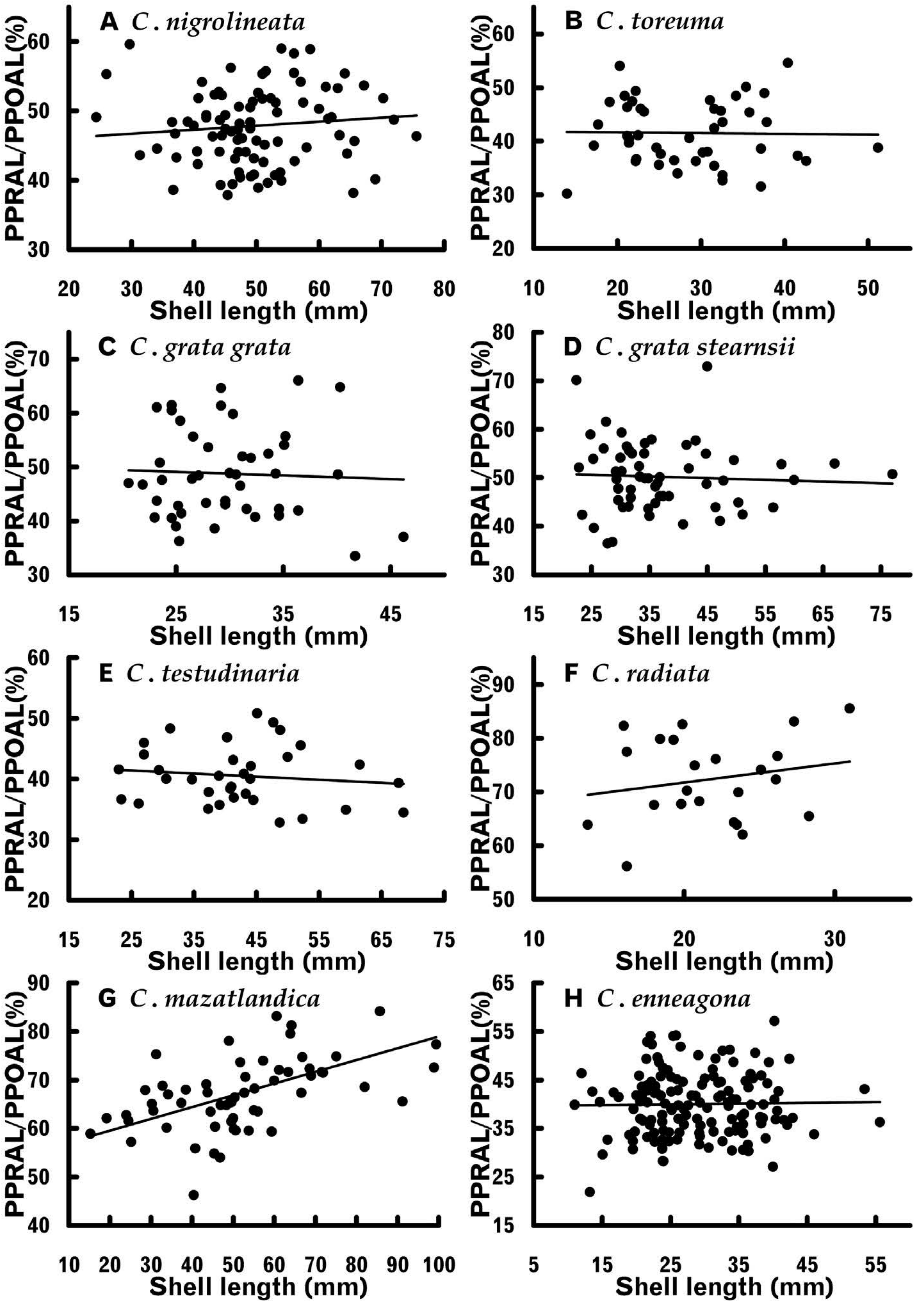

Fig. 8. Relationships between shell length and PPRAL/PPOAL ([projective pre-apex length]/[projective post-apex length]) proportions in Japanese nacellid limpets. Solid lines indicate linear regressions, whose parameters are referred to Table 1.

Despite considerable intraspecific color variation, however, the ICA color patterns were not related to growth in the other species and subspecies (Table 4). The ICA color patterns also differed between *Cellana grata grata* and *C. grata stearnsii*, *i.e.*, ICAs with dark orange (Fig. 3H) plus dark orange-inclusive complex patterns, which occurred in the former, did not appear in the latter, and those with white (Fig. 3L) plus white-inclusive complex patterns, which occurred in the latter, were absent in the former (Table 4).

Discussion

The present study revealed that many shell proportions in Japanese nacellids change with growth. In particular, it is remarkable that ratios of shell width (SWH/SL) and shell height (SH/SL) increase with growth in some species and subspecies (Figs. 4, 7; Table 1). The increase of these ratios infers simultaneous increase in shell volume, an inference consistent with the relative increase in shell weight (SW). Accordingly, the relative SW gain mainly may result from the relative increase in SWH and SH.

This is most significant in *Cellana nigrolineata*, in which the SWH/SL and SH/SL ratios apparently increase with growth (Figs. 4A, 5, 7A; Table 1) and the relative growth coefficient (*b* value) for SW is an extremely high at 3.350 (Table 3). This pattern is basically consistent with that in *C. mazatlandica,* although the *b* value is less than that in *C. nigrolineata* (Figs. 4G, 6, 7G; Tables 1, 3). While the *b* value in *C. testudinaria* significantly exceeded 3 (Table 3), the SWH/SL and SH/SL ratios were stable (Figs. 4E, 7E; Table 1). This infers that shells of this species become thicker with growth, causing the relative SW gain (Table 3). In *C. grata stearnsii*, although the SH/SL ratio shows a slight increase with growth (Fig. 7D, Table 1), it may be too weak to influence the relative SW gain. In *C. enneagona*, the SWH/SL ratio increases (Fig. 4H; Table 1), but the SH/SL ratio decreases (Fig. 7H; Table 1) and relative SW does not change (Table 3). This implies that rates of SWH/SL increase and SH/SL decrease may balance each other; if the rate of SH/SL decrease were more than that of SWH/SL increase, the shells would become thicker with growth. On the other hand, in *C. grata grata* and *C. radiata*, all the ratios, including SH/SL and SWH/SL, and the relative SW do not change with growth (Figs. 4F, 7F; Tables 1–3). This indicates isometric growth, unlike the other species and subspecies. Similarly, *C. toreuma*, in which growth-related changes were recognized only in the internal colored area dimensions (Tables 1, 2), showed a stable relationship for SL–SW (Table 3).

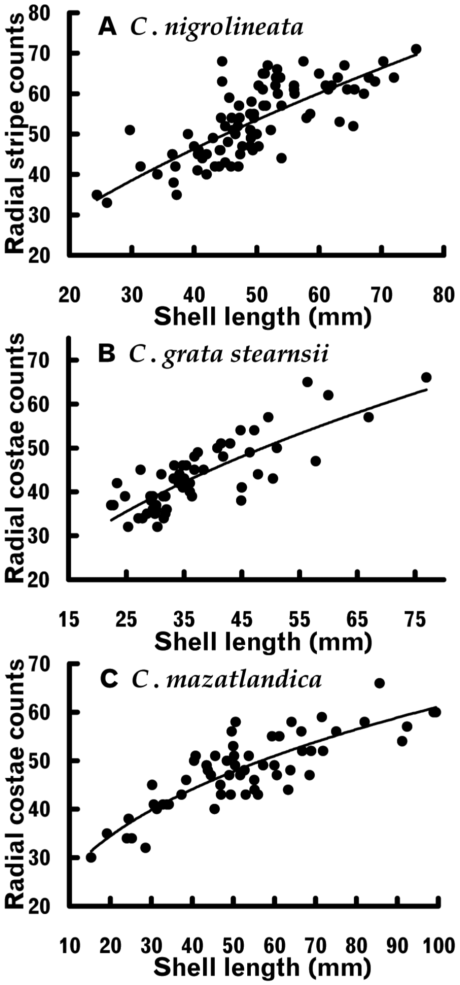

Fig. 9. Relationships between shell length (SL) and radial stripes counts (*Cellana nigrolineata*) and between SL and radial costae counts (*C. grata stearnsii* and *C. mazatlandica*). Solid lines indicate power regressions, whose parameters are referred to text.

Cabral (2007) examined the shells of four European Atlantic patellid limpets (*Patella intermedia*, *P. rustica*, *P. ulyssiponensis* and *P. vulgata*), detecting very similar species-specific growth-related proportion changes. Also, Vat (2000) carried out shell morphometry on *Patella granularis* from South Africa and found that the shells grow allometrically, increasing in SH more rapidly than in SL. These cases infer that such phenomena are common in the limpets of the order

Table 3. Results of calculations of regressions between shell length (SL) and shell weight (SW) ($\ln SW = a \ln SL + b$) and t tests to examine significance of parameter a for a null hypothesis ($a = 3$) for Japanese nacellid limpets.

Species or subspecies	*a*	*b*	*r*	*t*
C. nigrolineata	3.350	–11.351	0.984	4.946***
C. toreuma	3.089	–10.830	0.989	1.229
C. grata grata	2.935	–9.516	0.938	–0.392
C. grata stearnsii	3.090	–9.733	0.961	0.759
C. testudinaria	3.187	–10.482	0.987	2.064*
C. radiata	3.037	–9.683	0.963	0.143
C. mazatlandica	3.140	–10.140	0.991	2.539*
C. enneagona	2.961	–10.180	0.971	–0.623

Asterisks indicate significance of *t* vales; single and triple asterisks indicate 5% and 0.1% levels, respectively.

Patellogastropoda, necessitating further examinations for many other taxa of this order.

Despite very little such morphometric information for the Japanese nacellid limpets, some investigation has been performed for *C. mazatlandica*, because it is endemic to the Ogasawara Islands and has been designated as a natural monument. Kuwasawa *et al*. (1980) measured SL, SWH, SH and body weight (BW, weight of shell plus soft parts) for many *C. mazatlandica* individuals and expressed their relationships with the linear formulae (SWH/SL = 0.45, SH/SL = 0.83, BW/SL = 5.28). In the former two formulae, the right side numbers may have been transposed by mistake (see Figs. 4G, 7G). Anyway, it is not useful to apply linear formulae to the ratios of SWH/SL and SH/SL, because they apparently increase with growth (Figs. 4G, 7G; Table 1). It is also inappropriate to apply linear formula to SL–BW, because the SL–BW regressions in the nacellid limpets are expressed well with power formulae ($BW = a\,SL^b$) (Yokogawa, unpubl.), like the SL–SW regressions (Table 3). On the other hand, Yazaki (2009) noted that *C. mazatlandica* from Minami-jima, a small uninhabited island in the Ogasawara Islands, had relatively higher SH to specimens from the other Ogasawaran islands. This may be because the *C. mazatlandica* individuals measured from Minami-jima comprise mainly large-sized shells (Shigei, 1970; Horikoshi *et al*., 1997), in which the SH/SL proportions are greater (Figs. 6, 7G). Although the reason why shell size of the population in Minami-jima biases toward larger is uncertain, it may be based on difference of environmental factors such as a predation pressure from enemy animals including human.

Regarding the relative apex position, the apex is located near to the center of the shell in *C. radiata*, unlike in the other Japanese nacellids (Fig. 8), and this has been regarded as a character of this species. It was pointed out by Habe (1961) as useful in distinguishing this species from among its congeners in East Asia. Using this criterion, the Taiwanese "*Cellana radiata*" illustrated by Hu & Tao (1995) are not identified as this species, because the apex in their specimens is located forward. Unlike the other Japanese nacellid limpets, *C. radiata* shows isometric growth in all aspects, including the relative apex position (Tables 1, 2), inferring phylogenetic peculiarity of this species.

It is also notable that the apex position clearly shifts backward with growth in *C. mazatlandica*, unlike in the other Japanese nacellids. Although Habe (1975) noted that the apex was located near to the center of the shell in *C. mazatlandica*, this could be based on larger adult individuals. Among the four European patellid limpets cited earlier (Cabral, 2007), a similar growth-related apex shift was also recognized in *P. intermedia* and *P. vulgata*, implying the

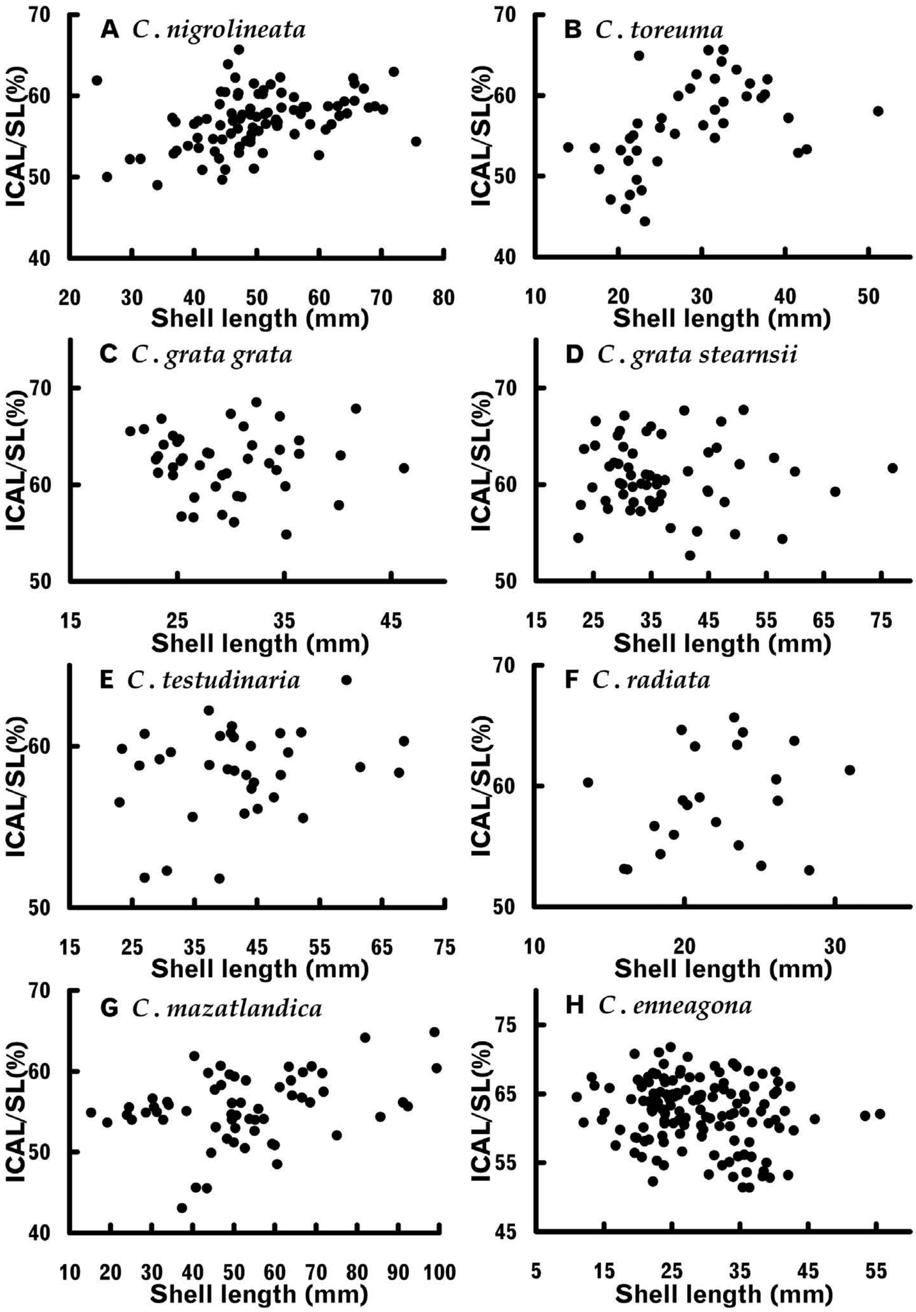

Fig. 10. Relationships between shell length and ICAL/SL ([Internal colored area length]/[Shell length]) proportions in Japanese nacellid limpets.

common occurrence of this phenomenon in the order Patellogastropoda. Whether or not this character emerges phylogenetically, it is a distinctive character among the Japanese nacellids. Because *C. mazatlandica* is endemic to the Ogasawara Islands, which are located far from the Japanese main islands (*e.g.*, Fukuda, 1993; Horikoshi *et al.*, 1997), it might have evolved distinct characters in the isolated environment.

Table 4. Growth-related frequency distributions of color patterns in internal colored areas for Japanese nacellid limpets.

Range of shell length (mm)	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100
Cellana nigrolineata									
Dark red		3	5	20	14	8	3		
Dark brown			1	11	2	2			
Light brown			1	5	4				
Dark brown/dark red			1	2	2	3			
Dark brown/light brown			1	2	3				
Cellana toreuma									
Light brown		8	3	1					
Light brown/white	1	6	9						
Dark brown			1						
White		4	3	1	1				
Colorless	3	2		1					
Cellana grata grata									
Dark brown		22	7	1					
Dark orange			5	1					
Light brown		1		1					
Dark brown/dark orange		3	4	1					
Cellana grata stearnsii									
Dark brown		13	12	3					
Light brown			4	3	3				
Dark brown/white		2	5						
Light brown/white			3	5	1	1			
White			1		1		1		
Cellana testudinaria									
Dark brown		6	5	4					
Dark brown/pale			2	8					
Pale				3	3	3			
Cellana radiata									
Orange	2	7							
Dark brown	3	1	1						
Dark brown/white	4	5							
Orange/white		1							
Cellana mazatlandica									
Dark brown	2	4	6	3	2				
Dark brown/cream			2	9	3	3			
Cream				3	8	6	3	2	4
Cellana enneagona									
Dark brown	7	23	5						
Brown		2		2					
Dark brown/white	5	40	28	8	1				
Brown/white	3	3	3	1					
White			8		1				

Two colors connected with a virgule indicate complexes of both colors.

Meristic characters such as radial stripes (RS) in *C. nigrolineata* and radial costae (RC) in *C. grata stearnsii* and *C. mazatlandica* all showed apparent growth-related increases (Fig. 9), which were revealed to be negatively allometric (Table 1). This is because initially narrow RS and RC newly form between the existing ones and become prominent with growth (Figs. 5, 6), and the degree of the new formation reduces with growth.

Horikoshi *et al*. (1997) described *C. mazatlandica* and cited RC counts of 25–35, although they mentioned growth-related occurrence of secondary RC between main RC. Regarding the SL–RC relationship (Fig. 9C), their description may have been based on smaller individuals (*ca*. SL 30–40 mm), not representing the full-sized individuals. As the phenomena of growth-related increase in meristic characters may be common in other nacellid limpets, descriptions should carefully consider these phenomena.

In reference to the internal colored area (ICA), ratios of its length (ICAL/SL) and width (ICAW/SL) increase with growth in some species but do not change in the remainder, indicating specific peculiarity (Fig. 10; Table 1). Although the ICA color pattern compositions vary by species and subspecies, apparent growth-related changes were observed in *C. testudinaria* and *C. mazatlandica* (Table 4). This phenomenon can be explained by the hypothesis that new light color layers (pale and cream in the former and latter, respectively) are gradually formed over the dark brown basal layers that are observed in smaller individuals. Formation of the new layers begins from the anterior part of the ICA (Fig. 3O, 3T), expanding to cover the entire base layer (Fig. 3N, 3S).

It is notable that only these two of the eight Japanese nacellid species show this growth-related color change in the ICA. It has been suggested that the two species are not closely related to each other genetically (Nakano & Ozawa, 2007; Nakano *et al*., 2009). This implies that such characters have emerged not phylogenetically but randomly during the evolution of nacellids.

The present study revealed that many conchological characters of the Japanese nacellid limpets change with growth, and that their patterns are distinct in different species. On the other hand, the suggestion that conchological characters are affected by environmental and genetic factors has been made in relation to some mollusks, including *Cellana nigrolineata* (Yokogawa, 2014), *Littorina obtusata* (Trussell & Nicklin, 2002), *Nucella lapillus* (Palmer, 1990) and *Ruditapes philippinarum* (Yokogawa, 1998), and examinations are needed to establish whether such factors affect even growth-related conchological changes. Nevertheless, though growth-related changes have hardly been considered for most other shell-bearing mollusks, they are important especially for taxonomic analyses in which the shell is carefully examined. In particular, such examinations for the other true limpets including those of the family Patellidae and Lottidae, which comprise lots of members, may give some valuable new information. These may well have significant implications for taxonomic analyses, and this emphasizes the need for greater accumulation of basic morphological information.

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日本産ヨメガカサガイ科貝類の成長に伴う殻形態の変化

横川浩治

要　約

日本産ヨメガカサガイ科貝類 7 種（マツバガイ，ヨメガカサ，ベッコウガサ，オオベッコウガサ，クルマガサ，カサガイ，シワガサ）と 1 亜種（アミガサ）について殻形態の成長に伴う変化を調べた。成長に伴う殻形態変化では，多くの種または亜種で殻径，殻高，内面着色域径などが成長に伴って相対的に大きくなり，特にマツバガイとカサガイでは殻のほとんどの部位で成長に伴う相対的増大がみられた。一方ベッコウガサとクルマガサでは殻の全部位で成長に伴う変化はなく，またシワガサでは殻高が成長に伴って相対的に小さくなることが特徴的であった。殻頂の相対的な位置はカサガイでは成長に伴って後方に移動するが，それ以外の種と亜種では殻頂の相対位置は個体サイズに関係なく一定であった。計数形質としてマツバガイの放射彩数およびアミガサとカサガイの放射肋数を調べたところ，これらすべてで成長に伴う顕著な増加がみられた。内面着色域の色彩は種ごとにかなり独自性がみられたが，オオベッコウガサとカサガイでは成長に伴う明瞭な変化がみられ，小型個体にみられる暗褐色の地色の上に別の色の新たな層が徐々に形成されていくものと考えられた。以上のように，日本産ヨメガカサガイ科貝類では成長に伴って殻の多くの形質が変化し，そのパターンには顕著な種特異性があることが明らかになった。