# ANATOMICAL AND MOLECULAR STUDIES REVEAL SEVERAL CRYPTIC SPECIES OF THE ENDEMIC GENUS *MANDARINA* (PULMONATA: HELICOIDEA) IN THE OGASAWARA ISLANDS

SATOSHI CHIBA[1] AND ANGUS DAVISON[1,2]

[1]*Graduate School of Life Science, Department of Ecology and Evolutionary Biology, Tohoku University, Aobayama, Sendai 980-8578, Japan;*
[2]*Institute of Genetics, School of Biology, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK*

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## ABSTRACT

The endemic genus *Mandarina* from the Ogasawara Islands in the north-western Pacific has undergone a significant adaptive radiation, and so is of interest in understanding speciation in land snails. While the majority of *Mandarina* species are easily recognized because underlying morphological differences in genital characters are mirrored by differences in the shell and ecology, we show here that the genus also includes several cryptic species. On the basis of anatomical characters described here, and supported by previously published phylogenies using mitochondrial rRNA sequences, we conclude that five distinct species were previously included in two nominal species: *Mandarina hayatoi* n. sp., and *M. kaguya* n. sp., both previously *M. hahajimana*, are a morphologically variable, allopatric/parapatric species complex on the Hahajima archipelago; *M. tomiyamai* n. sp. is conchologically similar to *M. hirasei* on Chichijima, but with distinct genital morphology. Populations of these cryptic species may have similar shells because of convergent evolution, a result of adaptation to similar arboreal lifestyle, so divergence of sexual organs must have occurred independently of habitat preference. These findings therefore support the assertion that sexual selection is an additional factor in the radiation of *Mandarina*.

## INTRODUCTION

The Ogasawara Islands comprise about 30 small volcanic islands in the North-west Pacific, about 1,000 km south of Japan, and have a biota that is characterized by endemism in plants, birds, insects and land snails. Of these, the radiation of the endemic land snail genus *Mandarina* is perhaps the most dramatic and also best studied (Chiba, 1999a, 2004; Davison & Chiba, 2006a, b). Twelve extant species have been described (Richardson *et al*., 1839; Pilsbry, 1890, 1894, 1901, 1902, 1903, 1928; Kuroda, 1930; Emura, 1943; Habe, 1973; Minato, 1978; Chiba, 1988, 1989).

Recent molecular analyses have shown that *Mandarina* is derived from a common ancestor with *Euhadra*, an endemic genus of the mainland of Japan (Davison & Chiba, 2006a). Upon arriving in the islands, the ancestor of *Mandarina* underwent diversification into arboreal and ground-living ecotypes, a process that may have occurred repeatedly on different islands (Chiba, 1999a; Davison & Chiba, 2006a), and was most likely promoted by character displacement among sympatric species (Chiba, 1996, 1999b; Chiba & Davison, 2007). To add to this complex pattern, species of similar ecotype sometimes abut and hybridize in regions of parapatry, forming complex spatial patterns of genetic and morphological variation (Davison & Chiba, 2006b).

Although the relationship between morphology and habitat use has been extensively studied in *Mandarina* species, their reproductive organs are poorly characterized. Accordingly, it is possible that several cryptic species have been overlooked. There is also an intriguing question as to the role of sexual selection in the radiation of *Mandarina*, if sexual organs evolve independently of shell morphology and are a potential isolating mechanism. In this study, we therefore investigate the genital morphology of two arboreal species of *Mandarina*, showing that genitalia are also sometimes divergent between separate populations, so leading us to describe several otherwise cryptic species.

Correspondence: S. Chiba; e-mail: schiba@biology.tohoku.ac.jp

Unfortunately, although the radiation of *Mandarina* is an excellent model system for understanding evolution and speciation, the genus is under threat because of introduced alien predators. Predatory flatworms, including *Platydemus monokwari* De Beauchamp, an important predator of land snails, and a predatory land snail *Euglandina rosea* (Férussac), have caused a marked decline in population density and distribution over the past 10 years (Kawakatsu *et al*., 1999; Tomiyama, 2002; Okochi *et al*., 2004; Sugiura *et al*., 2006). Thus, further ecological, evolutionary and taxonomic studies are urgently required from a conservation perspective, so as to inform any future captive-breeding and/or reintroduction programmes. Regrettably, it is likely that one of the new species, *Mandarina tomiyamai* is already extinct.

## MATERIAL AND METHODS

Samples of *Mandarina* used in the present study were collected from four localities in Chichijima, six in Hahajima and one each in Mukoujima, Imotojima, Anejima and Meijima (Fig. 1). All were collected between 2003 and 2006, except for three from Chichijima (Mikadukiyama, Tsurihama and Minamizaki) which were collected in 1990–1992. The material examined in this study has been deposited in the University Museum, the University of Tokyo (UMUT) and University Museum, Tohoku University (TUMC).

Adult shells were measured for whorl number (WH), shell height (*H*) and maximum diameter (*D*) using a digital micrometer. Relative shell height (RH) (height/diameter) was also calculated. Shells of 4–18 individuals were examined for each population. Specimens were drowned in water and preserved in 70% ethanol prior to anatomical study of the external shape and internal sculpture of the penis (Chiba, 1989). From three to five snails (all adults) were dissected from each population, and both the internal and external genital

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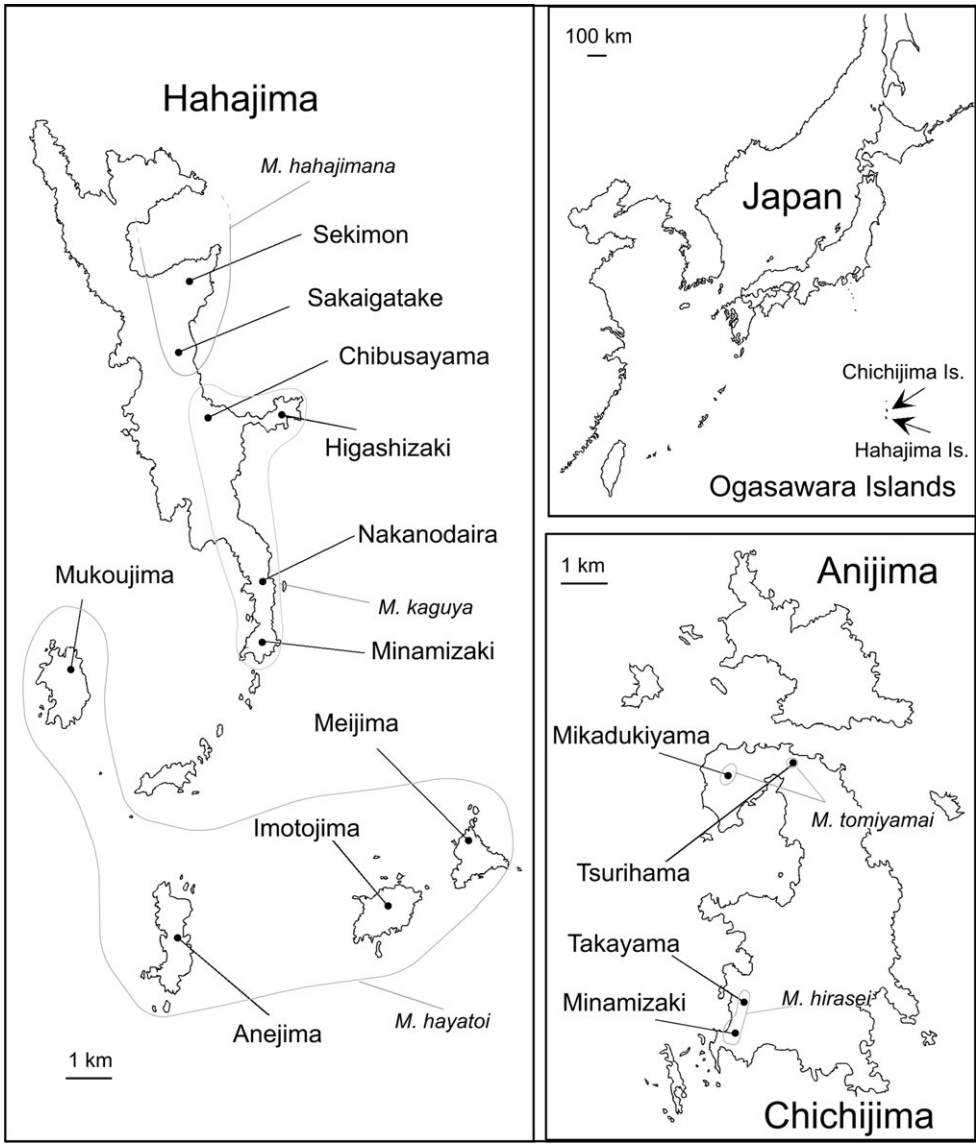


**Figure 1.** Map of Hahajima Islands and Chichijima Islands in the Ogasawara Islands, showing the sample locations. Estimated distribution ranges of the species are also shown.

morphology observed under a dissecting microscope. The genitalia were photographed for each specimen and eight measurements taken (Fig. 2), including the length of penis (PL), maximum thickness of penis (PT), length of epiphallus (EL), length of flagellum (FL), maximum thickness of flagellum (FT), length of vagina (VL), maximum thickness of vagina (VT) and length of bursa stalk (BL).

As phylogenetic data have previously been gathered on the species considered here, using mitochondrial 12S and 16S rRNA sequences (Davison & Chiba, 2006a), we consider the new descriptions in light of these relationships (Fig. 3).

## SYSTEMATIC DESCRIPTIONS

### Order Stylommatophora

### Family Bradybaenidae Pilsbry, 1934

### Genus *Mandarina* Pilsbry, 1894

*Type species*: *Helix mandarina* Gray, 1839 (by monotypy).

*Diagnosis*: Shell solid, extremely variable in shape and size; diameter from 15 mm to more than 80 mm. With or without umbilicus. Protoconch large, exceeding 1/5 of adult shell width. Many fine spiral striae on surface of the protoconch; teloconch surface generally smooth; aperture oval; lip thickened, expanded and reflected. Penis with thickened sheath but without penial appendix. Epiphallus generally long and narrow. Dart sac, mucus gland and bursa duct diverticulum lacking.

### *Mandarina kaguya* new species
(Figs 4A–C, 6A, B)

*Mandarina hirasei*—Maeda *et al*., 1987: 33, fig. 286.
*Mandarina hahajimana*—Maeda *et al*., 1987: 33, fig. 285b.
Chiba, 1989: 241, figs. 11–14. Chiba, 1999a: 469, fig. 6.

*Types*: Holotype: UMUT RM29208, summit of Chibusayama, Hahajima, Ogasawara (D: 18.4 mm; H: 14.5 mm; WH: 2.7). Paratypes: UMUT RM29209 ($n = 1$), Minamizaki, Hahajima, Ogasawara; UMUT RM18464 ($n = 6$), Minamizaki, Hahajima, Ogasawara; TUMC 24503 ($n = 1$), Higashizaki,

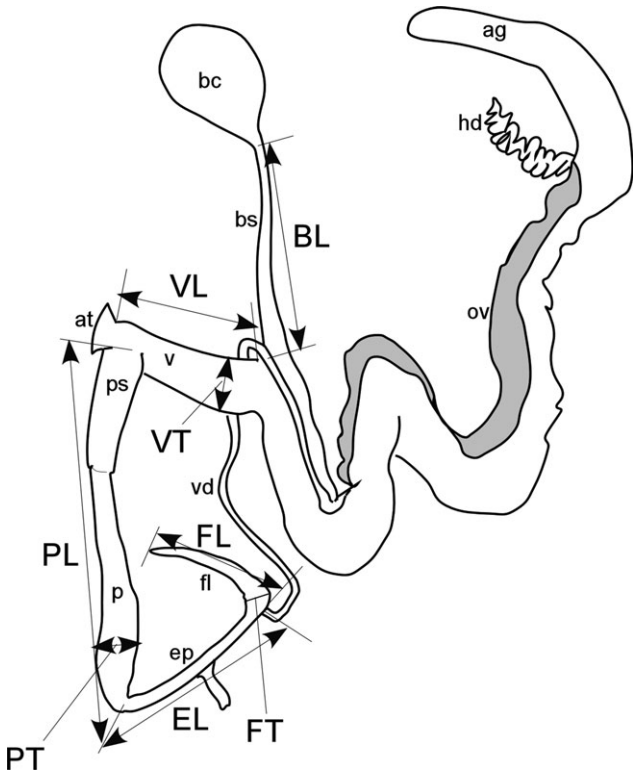


**Figure 2.** Measurements of the genitalia of *Mandarina*. Measurements: PL, length of penis; PT, maximum thickness of penis; EL, length of epiphallus; FL, length of flagellum; FT, maximum thickness of flagellum; VL, length of vagina; VT, maximum thickness of vagina; BL, length of bursa stalk. Abbreviations: at, atrium; ag, albumen gland; hd, hermaphroditic duct; bc, bursa copulatrix sac; bs, bursa stalk; ep, epiphallus; fl, flagellum; ov, oviduct; p, penis; ps, penial sheath; pp, pilaster; pv, verge; v, vagina; vd, vas deferens.

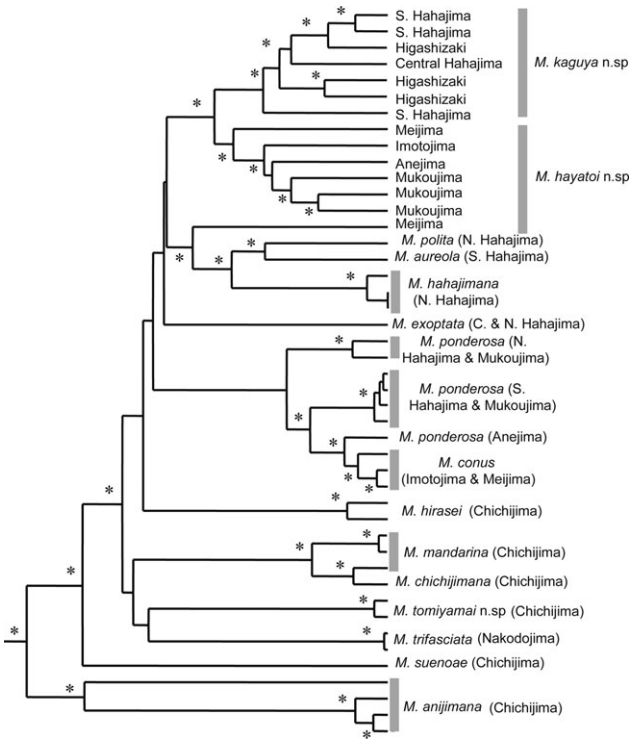


**Figure 3.** Phylogenetic relationship of the species of *Mandarina* in the Ogasawara Islands. The phylogeny was estimated from mitochondrial 16SrRNA and 12SrRNA sequences (redrawn from Davison & Chiba, 2006a). Branches supported by bootstrap values >70% are indicated by an asterisk.

Hahajima, Ogasawara; TUMC 24504 ($n = 10$), Higashizaki, Hahajima, Ogasawara; UMUT RM18461 ($n = 10$), Chibusayama, Hahajima, Ogasawara; UMUT RM18463 ($n = 4$), Nakanodaira, Hahajima, Ogasawara.

*Etymology*: The species is named after 'Kaguya-hime', a princess in a Japanese folk tale.

*Diagnosis*: Shell medium to small, globular or flattened; body whorl with rounded or barely angulated periphery. Penis three-times vaginal length. Flagellum short. Epiphallus uniformly slender and short; bursa stalk short. Upper penial wall ornamented with more than 10 regularly wavy pilasters. Verge small and rounded.

*Shell* (Fig. 4A–C): Shape and size variable, 16.2–23.5 mm in diameter and 11.5–17 mm in height. Relative shell height from 0.61 to 0.86. Spire strongly (Chibusayama and Higashizaki) or weakly (Minamizaki and Nakanodaira) domed. background colouring yellow, white, orange, purple, brown or bright green, commonly with banding completely missing or only present as narrow black or reddish-brown bands above, or both above and below periphery. Body whorl with rounded or barely angulated periphery. Suture moderately to strongly impressed. Embryonic whorls 1.5–2.0; first whorl smooth, thereafter with several fine spiral striae. Surface of periostracum of post-embryonic shell appearing smooth and slightly shiny, but with numerous very fine spiral lines and fine radial growth lines. Umbilicus closed in populations from Higashizaki but clearly open in shells from southern Hahajima (Minamizaki), typically slightly open in Chibusayama specimens, but occasionally closed by reflected columellar lip. Base of shell rather flat and somewhat excavated around umbilicus in shells from Minamizaki, rounded and convex in those from Higashizaki. Aperture round in shells from Chibusayama and Higashizaki, but wider than high and narrowed at base in other localities. Lip outwardly expanded above periphery, reflected below periphery. Apertural lip, columella, and parietal callus white, purple or brownish. Slightly thickened parietal callus extending slightly to left of columella in basal view. Teloconch whorls 2.5–3.2.

*Anatomy* (Fig. 6A, B): Atrium short. Penis thick relative to other species and three-times vaginal length (mean PL = 22.6 mm, mean PT = 2.45 mm, mean VL = 8.14 mm, Chibusayama; mean PL = 30.1 mm, mean PT = 3.25 mm, VL = 10.4 mm, Higashizaki; mean PL = 25.3 mm, mean PT = 3.09 mm, mean VL = 8.70 mm, Nakanodaira; mean PL = 24.7 mm, mean PT = 3.04 mm, mean VL = 8.52 mm, Minamizaki). Penis slightly thickened and covered by sheath at base, narrowed at middle and gradually thickened toward top (Fig. 6A). Flagellum short with sharply pointed tip (mean FL, 4.07 mm for Chibusayama, 6.28 mm for Higashizaki, 5.10 mm for Nakanodaira and 4.98 mm for Minamizaki). Epiphallus uniformly slender and short relative to other species, less than half penial length (mean EL, 8.92 mm for Chibusayama, 12.0 mm for Higashizaki, 9.58 mm for Nakanodaira and 9.44 mm for Minamizaki). Penial retractor muscle long, thickened, inserting near proximal end of epiphallus. Vas deferens long and convoluted, ending in dilated penial atrium. Vagina cylindrical, relatively short and 1.5 times thickness of penis (mean VT, 3.56 mm for Chibusayama, 4.76 mm for Higashizaki, 4.50 mm for Nakanodaira and 4.41 mm for Minamizaki). Oviduct enlarged and compressed, fused prostate located below oviduct. Albumen gland curved

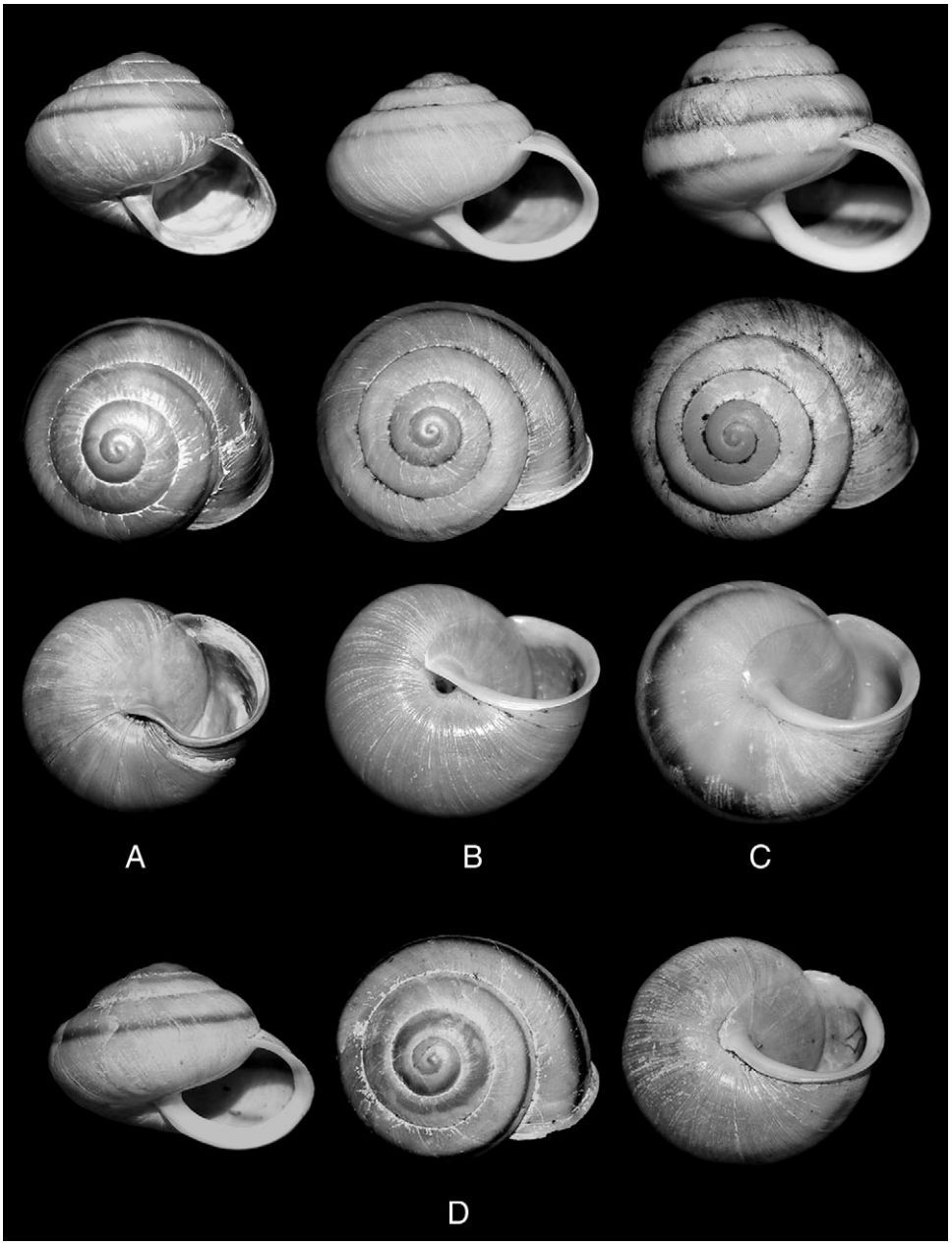


**Figure 4.** Shells of *Mandarina*. **A.** *M. kaguya*, Chibusayama (UMUT RM29208). **B.** *M. kaguya*, Minamizaki (UMUT RM29209). **C.** *M. kaguya*, Higashizaki (TUMC 24503). **D.** *M. hahajimana*, Sakaigatake (UMUT RM18460).

and lingulate. Bursa copulatrix oval. Bursa stalk short and uniformly narrow (mean BL, 11.2 mm for Chibusayama, 12.5 mm for Higashizaki, 11.8 mm for Nakanodaira and 11.8 mm for Minamizaki). Internally penial wall sculpted with approximately eight rows of regularly folded, separate, uniformly sized pilasters at base and middle (Fig. 6B). Upper penial wall ornamented with more than 10 columns of separate, regularly undulating pilasters. Verge small, round, and sculptured with weak, regular, longitudinal pilasters.

*Distribution* (Fig. 1): Known from central (Chibusayama) to the southernmost part of Hahajima (Minamizaki). In Minamizaki and Nakanodaira it lives on pandanus and palm trees, and occasionally on the ground near the roots of trees, but in Higashizaki it has only been found living on the ground.

*Remarks*: *Mandarina kaguya* was classified as *M. hahajimana* or *M. hirasei* in previous studies because of the close resemblance in shell morphology, confused by the fact that both are variable. However, in *M. kaguya* the penis is twice as thick, and the bursa stalk and epiphallus shorter than *M. hahajimana* (mean PT = 1.17 mm, mean BL = 21.5 mm, mean EL = 13.5 mm, Sakaigatake, UMUT RM18460; mean PT = 1.45 mm, mean BL = 21.8 mm, mean EL = 13.9 mm, Sekimon, UMUT RM18459) (Fig. 6E) and *M. hirasei* (mean PT = 1.35 mm, mean BL = 26.2 mm, mean EL = 13.4 mm, Takayama, UMUT RM18423; mean PT = 1.33 mm, mean BL = 26.0 mm, mean EL = 13.4 mm, Minamizaki, UMUT RM18424) (Fig. 7C). Internally, *M. kaguya* has a penis with a greater number of pilasters than *M. hahajimana* (Fig. 6F) and *M. hirasei* (Fig. 7D): the upper part of the penis of *M. hahajimana* and

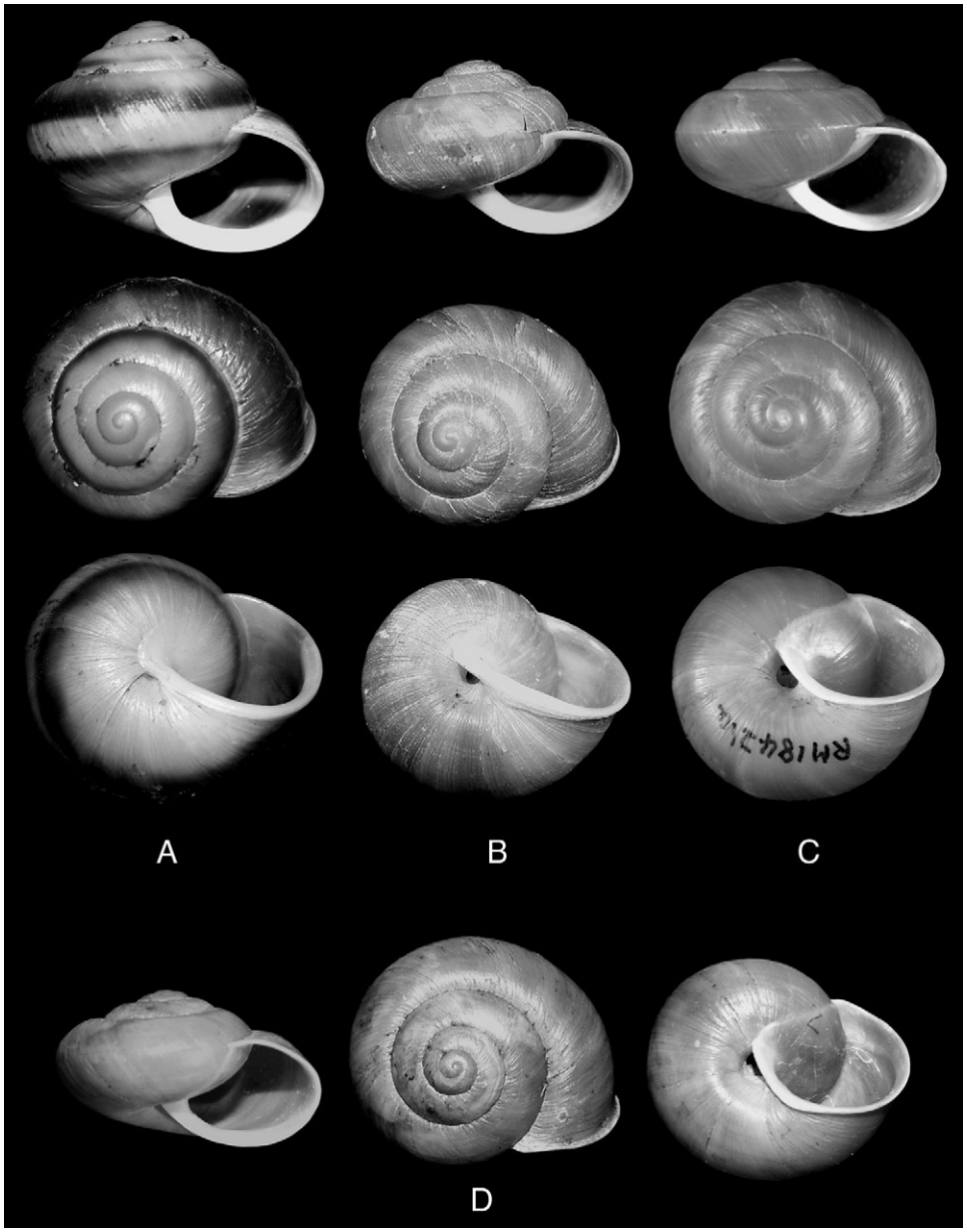


**Figure 5.** Shells of *Mandarina*. **A.** *M. hayatoi*, Imotojima (TUMC 24987). **B.** *M. hayatoi*, Mukoujima (TUMC 24989). **C.** *M. tomiyamai*, Mikadukiyama (UMUT RM18421a). **D.** *M. hirasei*, Takayama (RM18423a).

*M. hirasei* has less than eight pilasters, whereas *M. kaguya* has more than 10. In addition, the pilasters of *M. kaguya* fold tightly and regularly, but those of *M. hahajimana* are nearly linear and only slightly wavy (Fig. 6B). The verge of *M. kaguya* is small but obvious, whereas that of *M. hahajimana* is almost missing.

Although there is no clear geographical variation in genital morphology within species, considerable geographical variation exists in shell morphology. The spire is flatter in the Minamizaki (mean RH = 0.73) and Nakanodaira (mean RH = 0.70) populations than at Chibusayama (mean RH = 0.78). In addition, the umbilicus in the two former populations is much more obvious than that of the last. Shells of these populations are very similar to those of *M. hirasei*, and are often indistinguishable. The Higashizaki population possesses a larger shell (mean $D = 22.3$ mm) without an umbilicus, that is quite similar to the shell of *M. hayatoi* from Imotojima and Meijima.

Shell shape, size, surface sculpture and colour patterns of *M. kaguya* from Chibusayama (mean D = 19.5 mm, mean H = 15.1 mm and mean RH = 0.78) are very similar to those of *M. hahajimana* from Sakaigatake (UMUT RM18460, $n = 10$, mean D = 19.8 mm, mean H = 15.3 mm and mean RH = 0.77), and the differences are not statistically significant (MANOVA, $F = 0.35$, $P > 0.05$). However, these populations are still distinct, because *M. kaguya* from Chibusayama generally possesses a very small umbilicus (sometimes largely covered by an extended columellar lip), whereas *M. hahajimana* lacks an umbilicus (Fig. 4D). Because *M. hahajimana* and the Chibusayama population of *M. kaguya* both live on trees, and are never found on the ground, the similar shell morphology between the arboreal population of *M. kaguya* and *M. hahajimana* may be a result of convergence.

The phylogenetic relationship inferred from mitochondrial rRNA sequences suggests that *M. hahajimana* and *M. hirasei* are

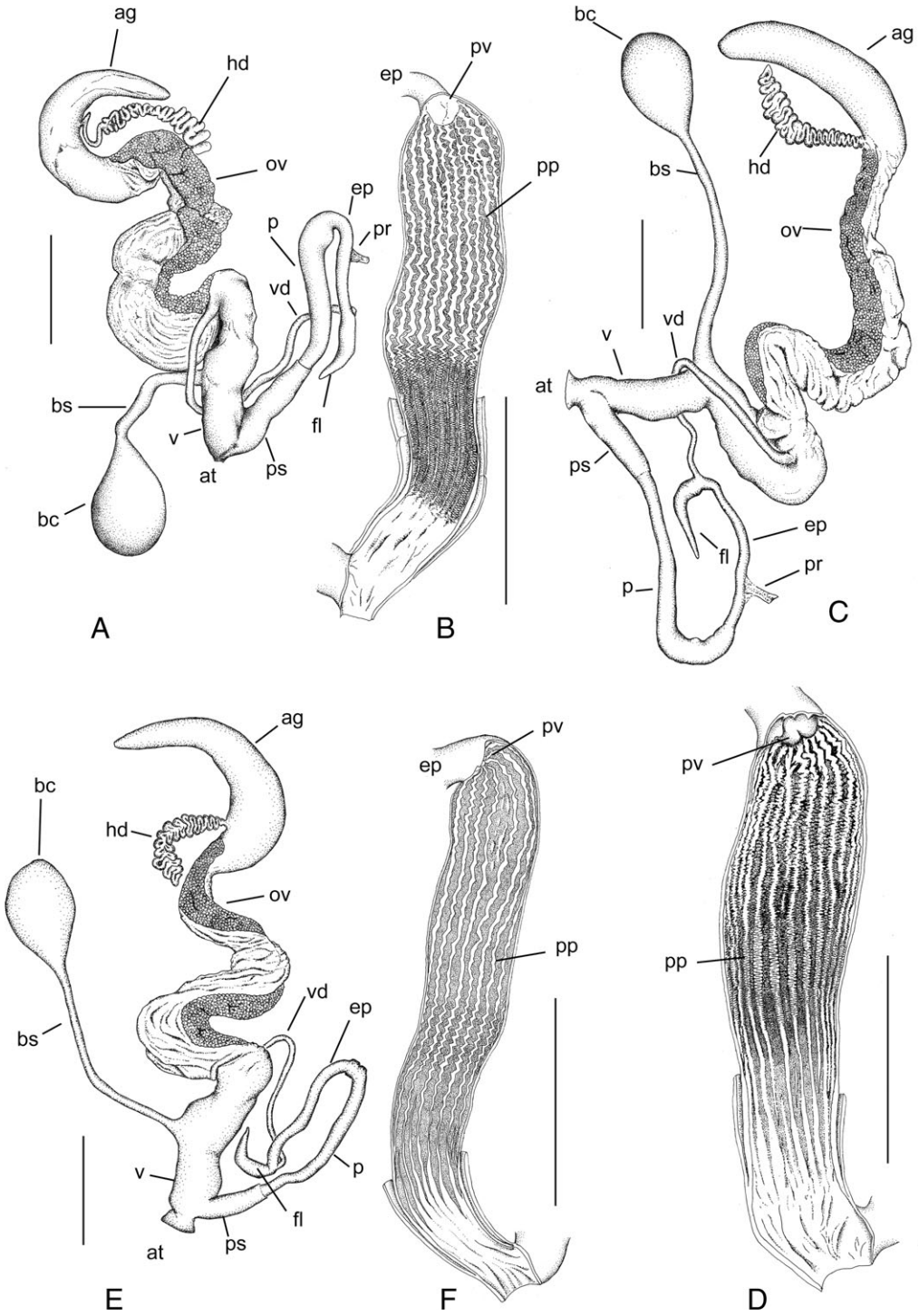


**Figure 6.** External genital morphology and internal penial ornamentation of *Mandarina*. **A, B.** *M. kaguya*. **C, D.** *M. hayatoi*. **E, F.** *M. hahajimana*. For abbreviations see Fig. 2. Scale bars = 10 mm.

distinct from *M. kaguya* (Fig. 3), supporting their discrimination as distinct species.

### ***Mandarina hayatoi* new species**
(Figs 5A, B, 6C, D)

*Mandarina hahajimana*—Tomiyama & Kurozumi, 1992: 50.
Chiba, 1999a: 469, fig. 6.

*Types*: Holotype: TUMC 24987, Imotojima, Ogasawara (D: 21.9 mm; H: 17.4 mm; WH: 2.7). Paratypes: TUMC 24991 ($n = 10$), Imotojima, Ogasawara; TUMC 24988 ($n = 1$), Meijima, Ogasawara; TUMC 24992 ($n = 7$), Meijima, Ogasawara; TUMC 24989 ($n = 1$) Mukoujima, Ogasawara; TUMC 24993 ($n = 5$), Mukoujima, Ogasawara; TUMC 24990 ($n = 1$), Anejima, Ogasawara; TUMC 24994 ($n = 5$), Anejima, Ogasawara.

*Etymology*: The species name is dedicated to Hayato Chiba (no relation), who assisted with the field trips for collecting material of this species.

*Diagnosis*: Shell medium-sized, globular or somewhat flattened; body whorl with rounded periphery. Penis approximately three-times vaginal length. Flagellum relatively long. Internal penial wall with approximately 8–10 rows of regularly and tightly folded, distinct, uniform pilasters in basal and middle portions; upper penial wall ornamented with more than 15 irregularly and tightly folded pilasters which merge. Verge small, round.

*Shell* (Fig. 5A, B): Medium-sized, 20.1–24.4 mm in diameter and 15.0–21.8 mm in height. Shape globular with spire strongly domed in populations from Imotojima and Meijima (RH = 0.76–0.84), but flattened in populations of Mukoujima (RH = 0.65–0.74). Background colour yellow or brown, with black bands on the upper and lower parts of the periphery, or completely missing; populations from Imotojima and Meijima generally having two wide bands above and below periphery. Body whorl with rounded periphery. Suture moderately to strongly impressed. Embryonic whorls 1.5–2.3; smooth for first complete whorl, thereafter with a number of fine spiral lines on surface. Periostracum of post-embryonic shell appearing smooth and somewhat shiny but with numerous very fine spiral and radial growth lines. Umbilicus closed in Imotojima and Meijima populations, but open in those from Mukoujima. Shells from Amejima with open or closed umbilicus. Base of shell rather flat and somewhat excavated around umbilicus in Mukoujima, but rounded and convex in populations from Imotojima and Meijima. Aperture round in the Imotojima and Meijima populations, but wider than high in those from Mukoujima. Lip outwardly expanded above periphery and reflected below. Apertural lip, columella and parietal callus white or purple, extending slightly to left of columella in basal view; thin in Mukojima population, but slightly thickened in Imotojima and Meijima. Teloconch whorls 2.5–3.0.

*Anatomy* (Fig. 6C, D): Penis fairly long, cylindrical in shape, approximately three-times vaginal length (mean PL = 28.8 mm, mean VL = 8.92 mm, Mukoujima; mean PL = 30.3 mm, mean VL = 11.4 mm, Imotojima; mean PL = 30.0 mm, mean VL = 10.9 mm, Meijima; mean PL = 28.9 mm, mean VL = 10.3 mm, Anejima). Penis covered by sheath at base, narrowed at middle and gradually thickened toward upper part (Fig. 6C). Flagellum relatively long (mean FL, 8.70 mm for Mukoujima, 8.77 mm for Imotojima, 8.80 mm for Meijima, 8.89 mm for Anejima). Epiphallus approximately half penis length (mean EL, 13.6 mm for Mukoujima; 14.2 mm for Imotojima, 14.2 mm for Meijima; 13.8 mm for Anejima). Vagina cylindrical in shape, 1.5 times thickness of penis (mean VT = 2.92 mm, mean PT = 2.13 mm, Mukoujima; mean VT = 3.23 mm, mean PT = 2.22 mm, Imotojima; mean VT = 3.15 mm, mean PT = 2.18 mm, Meijima; mean VT = 3.04 mm, mean PT = 2.13 mm, Anejima). Bursa stalk long, usually same length as penis (mean BL, 27.3 mm for Mukoujima, 28.0 mm for Imotojima, 28.2 mm for Meijima, 27.5 mm for Anejima). Internally, penial wall sculpted by approximately 8–10 rows of regularly and tightly folded, separate, equal-sized pilasters at base/middle (Fig. 6D). Upper penial wall ornamented with more than 15 irregularly and tightly folded longitudinal pilasters which merge with each other. Verge small, round. Otherwise as for *M. kaguya*.

*Distribution* (Fig. 1): Imotojima, Meijima, Mukoujima and Anejima. Usually arboreal, but in Anejima also found living on the ground.

*Remarks*: This species was initially treated as a variant of *M. hahajimana*. However, it differs from *hahajimana* in having a much thicker penis (greater PT), larger verge and greater number of pilasters that are more tightly folded and merge into each other. The external morphology of the penis of *M. hayatoi* is similar to that of *M. kaguya*. However, *M. hayatoi* can be distinguished from *M. kaguya* by its more slender penis and flagellum and longer bursa stalk (greater BL). Internally, *hayatoi* differs in having a larger number of more complex pilasters (Fig. 6D); those of *M. kaguya* do not merge, and are more loosely and regularly folded (Fig. 6B).

Although no clear difference was found in genital morphology between the populations of *M. hayatoi* on different islands, there are two conchological types. The first has a larger, higher shell without an umbilicus (Fig. 5A) and is generally ground-dwelling; the second type has a smaller and flatter shell with an umbilicus (Fig. 5B) and is generally arboreal. All of the snails found in Meijima and Imotojima are of the first type, and those in Mukoujima are the second type; both types are found in Anejima. Despite the large morphological differences between the different types, the mitochondrial DNA sequences are monophyletic, except for a rare, deeply divergent lineage recovered from Meijima (Fig. 3).

### ***Mandarina tomiyamai*** **new species**
(Figs 5C, 7A, B)

*Mandarina hirasei*—Minato, 1978: 41, 42, 48–49, figs 1, 2.
Chiba, 1989: 234–235, fig. 8–3. Chiba, 1999a: 469, fig. 6.

*Types*: Holotype: UMUT RM18421a, Mikadukiyama, Chichijima, Ogasawara (D: 21.1 mm; H: 13.4 mm; WH: 2.5). Paratypes: UMUT RM18421b-r ($n = 17$), Mikadukiyama, Chichijima, Ogasawara; TUMC 24995 ($n = 5$), Tsurihama, Chichijima, Ogasawara.

*Etymology*: The species name is dedicated to Kiyonori Tomiyama, who collected the material used here.

*Diagnosis*: Shell small, flattened; umbilicus open; periphery of body whorl weakly angulated. Penis approximately twice vaginal length. Flagellum long. Upper and middle parts of penial wall ornamented with six to eight rows of straight or weakly undulating pilasters. Verge large, conical.

*Shell* (Fig. 5C): Small, 20.2–22.9 mm in diameter and 13.0–16.1 mm in height. Spire flattened (RH = 0.60–0.69). Colour brown, lacking bands. Body whorl with weakly angulated periphery. Suture moderately impressed. Embryonic whorls 1.7–2.2; smooth for first complete whorl, thereafter with several fine spiral striae. Surface of periostracum of post embryonic shell appearing smooth and shiny but with numerous very fine spiral lines and fine radial growth lines. Umbilicus deep and widely open. Base of shell rather flat and excavated around umbilicus. Aperture wider than high and narrowed at base. Lip outwardly expanded above periphery, reflected below. Apertural lip purple, but columella and parietal callus white; latter slightly thickened and extending slightly to left of columella in basal view. Teloconch whorls 2.3–2.

*Anatomy* (Fig. 7A, B): Penis long, cylindrical in shape, approximately twice vaginal length (mean PL = 22.9 mm, mean VL = 11.5 mm, Mikadukiyama; mean PL = 21.5 mm, mean VL = 11.0 mm, Tsurihama); penis narrow, slightly thickened and covered by sheath at the base, and uniformly slender thereafter (mean PT, 1.00 mm for Mikadukiyama, 0.98 mm for Tsurihama) (Fig. 7A). Flagellum long, slender with sharp tip (mean FL, 8.06 mm for Mikadukiyama, 7.65 mm for

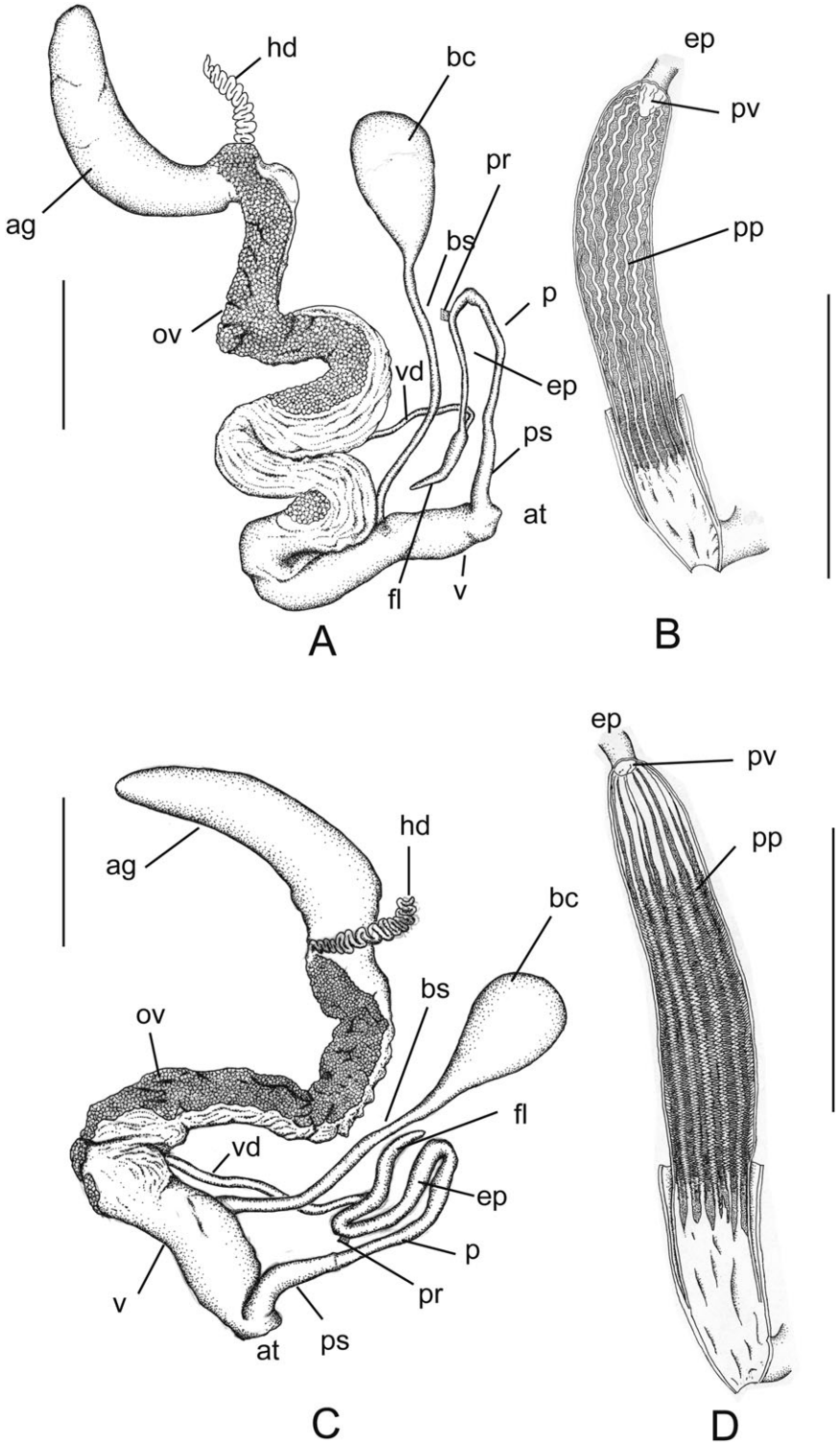


**Figure 7.** External morphology of the genitalia of *M. tomiyamai* (**A**) and *M. hirasei* (**C**) and internal ornamentation of penis of *M. tomiyamai* (**B**) and *M. hirasei* (**D**). For abbreviations see Fig. 2. Scale bars = 10 mm.

Tsurihama). Epiphallus narrow, approximately 2/3 PL (mean EL, 13.8 mm for Mikadukiyama, 13.0 mm for Tsurihama). Penial retractor muscle long, thickened, inserting near proximal end of epiphallus. Vas deferens long and convoluted, ending in dilated penial atrium. Vagina cylindrical in shape. Bursa stalk long, typically same length as penis (mean BL, 24.1 mm for Mikadukiyama, 24.3 mm for Tsurihama). Internal penial wall sculpted with 5–6 rows of regularly folded pilasters at base, but by 6–8 rows of straight or slightly wavy pilasters at the upper/middle part (Fig. 7B). Verge large, conical. Otherwise as for *M. kaguya*.

*Distribution* (Fig. 1): Northeastern Chichijima (Mikazukiyama and Tsurihama), where it lives on pandanus and palm trees.

*Remarks*: This species was initially ascribed to *Mandarina hirasei* because of the similarity in shell morphology, but differs in having a thinner penis (lower PT) with nearly straight pilasters; indeed the penis of *M. tomiyamai* is the thinnest of all *Mandarina* species. The pilasters of *M. hirasei* (Takayama, UMUT RM18423; Minamizaki, UMUT RM18424) are tightly folded at the middle and upper portions, whereas those of *M. tomiyamai* are not folded in these areas (Fig. 7B). In addition, *M. tomiyamai* possesses a large, conical-shaped verge, whereas that of *M. hirasei* is small and rounded. *M. tomiyamai* has a very similar shell to *M. hirasei*; although the former does tend to have a slightly stronger peripheral angle and smoother surface. The shell of *M. hirasei* (Takayama, UMUT RM18423, $N = 4$; Minamizaki, UMUT RM18424, $N = 22$) sometimes has bands on the upper and lower parts of the periphery, whereas that of *M. tomiyamai* is always unbanded.

Despite their closely similar shell morphology (Fig. 3), both the phylogenetic relationship inferred from mitochondrial rRNA sequences and the differences in their genitalia suggest that *M. tomiyamai* and *M. hirasei* are distinct species.

Sadly, *M. tomiyamai* may now be extinct as a result of predation by the introduced flatworm *Platydemus manokwari*.

## DISCUSSION

The present investigation of genital morphology in *Mandarina* shows that five cryptic species have been included in two nominal species, and this conclusion is supported by previously published phylogenies using mitochondrial rRNA. Large interspecific differences were found in the thickness and PL, and in the number and shape of pilasters on the internal wall of penis (Figs 6, 7), in contrast to the similarity in their shell morphology. Because the species are mostly arboreal and thus ecologically similar to one another, the difference in genital morphology among the species cannot be related to habitat use, but the similarity in shell morphology must in some part be due to convergent evolution. For example, on the basis of mitochondrial DNA sequences, *M. kaguya*, *M. tomiyamai* and *M. hirasei* are phylogenetically distantly related to each other, yet large differences in genital morphology are apparent between the sister taxa *M. kaguya* and *M. hayatoi* (Fig. 3). In contrast, a lack of variation in the genitalia among the conspecific populations contrasts with frequently high conchological and ecological diversity (e.g. *M. hayatoi*, Fig. 4A–C), suggesting that divergence of genital morphology occurs independently of divergence for habitat use and shell morphology.

Whether difference in genital morphology is an isolating mechanism in snails is a controversial and understudied subject. The little evidence that exists suggests that length differences between the organs that produce and receive the spermatophore are most likely to cause reproductive isolation (Madec & Guiller, 1993; Jordaens *et al*., 2002; Van Riel *et al*., 2003). Although details of the function of genital morphology are unclear in *Mandarina*, diversification in the reproductive anatomy (e.g. penis size and shape) of simultaneous hermaphrodites is presumably the result of sexual selection (Emberton, 1988; Michiels & Newman, 1998; Karlsson & Haase, 2002; Koene & Schulenburg, 2005; Schilthuizen, 2005). If correct, then this perhaps suggests that sexual selection is an added, hitherto unconsidered factor in the radiation of *Mandarina*. Although further detailed analyses of genitalia are needed, the present report of several cryptic species with distinct genitalia shows that *Mandarina* may be an excellent model system to address the issue of sexual selection in snails.

Finally, as the genital anatomy has been investigated for only a small number of populations in *Mandarina*, even in the case of the ground-dwelling species, the present findings suggest that there may be further unrecognized cryptic species of *Mandarina*. Since many populations of *Mandarina* are endangered, investigations of cryptic species based on genital anatomy and molecular analysis are crucial not only for identifying significant units of conservation unit, but also for developing a captive-breeding programme.

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