

Host specificity of parasitic gastropods of the genus *Annulobalcis* Habe, 1965 (Mollusca, Gastropoda, Eulimidae) from crinoids in Vietnam, with descriptions of four new species

Polina Y. DGEBUADZE
Alexander E. FEDOSOV
Yuri I. KANTOR

A. N. Severtzov Institute of Ecology and Evolution,
Russian Academy of Sciences,
Leninskiy prospect 33, Moscow 119071 (Russia)
p.dgebuadze@gmail.com

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ABSTRACT

Parasitic gastropods of the genus *Annulobalcis* Habe, 1965 (family Eulimidae), associated with the crinoids (comatulids) are common in Nhatrang Bay (Southern Vietnam). Morphological examination of *Annulobalcis* specimens, collected in this area revealed four distinct forms, significantly different also in host specificity. Mitochondrial Cox1 sequence data have shown these forms to comprise four distinct species, their relationships are discussed. As a result of morphological and molecular studies, four new species of the genus *Annulobalcis* are described: *A. albus* n. sp., *A. wareni* n. sp., *A. maculatus* n. sp., and *A. vinarius* n. sp. The three former species appeared to be strictly species specific in their choice of host (parasitizing a single host species each), while the latter was found on a number of hosts, although mostly on a single species. Speciation in relation to host specificity of *Annulobalcis* is discussed.

KEY WORDS

Mollusca,
Gastropoda,
Eulimidae,
Annulobalcis,
Echinodermata,
Crinoidea,
Vietnam,
parasitic,
new species.

RÉSUMÉ

La spécificité des gastéropodes du genre Annulobalcis Habe, 1965 (Gastropoda, Eulimidae) parasites de crinoïdes du Vietnam, avec la description de quatre nouvelles espèces.

Les gastéropodes parasites du genre *Annulobalcis* Habe, 1965 (famille Eulimidae), associés aux crinoïdes (comatules), sont communs dans la baie de Nhatrang (Sud Vietnam). Leur étude morphologique a révélé quatre formes distinctes, qui diffèrent également dans leur spécificité à l'hôte. Les séquences du gène mitochondrial Cox1 ont montré que ces formes correspondent à quatre espèces distinctes, dont les relations sont discutées ici. Sur la base de ces résultats morphologiques et moléculaires, quatre nouvelles espèces sont décrites: *Annulobalcis albus* n. sp., *A. wareni* n. sp., *A. maculatus* n. sp., et *A. vinarius* n. sp. Les trois premières ne sont présentes chacune que sur un seul hôte, tandis qu'*A. vinarius* a été trouvée sur plusieurs hôtes, bien que préférentiellement sur un seul. La spéciation d'*Annulobalcis* est discutée dans le contexte de cette spécificité vis à vis des hôtes.

MOTS CLÉS

Mollusca,
Gastropoda,
Eulimidae,
Annulobalcis,
Echinodermata,
Crinoidea,
Vietnam,
parasitisme,
espèces nouvelles.

INTRODUCTION

The family Eulimidae encompasses about 850 Recent described species and a large proportion of undescribed ones. Eulimidae are thought to be exclusively symbiotic associated with all major groups of echinoderms (Warén 1983; Jangoux 1990). Recent extensive biodiversity surveys, conducted by the Muséum national d'Histoire naturelle (Paris) have shown Eulimidae to be one of the five most diverse and abundant families of tropical marine molluscs (Bouchet *et al.* 2002).

Diversity of Eulimidae in regional check lists is often grossly underestimated, basically due to their small size (most Eulimidae species do not exceed 5 mm in length) and difficulties in collecting (especially in association with their hosts). Consequently this important family is often poorly represented in zoological collections: specialized techniques developed to cover gastropods of the smallest size were established mostly in last decades and are still rarely employed in field collecting.

Most eulimids possess a glossy very weakly sculptured or smooth shell and a simple uniform aperture. Unusual species richness and general uniformity in shell structure with paucity of informative morphologic characters throughout the family make the group extremely hard for taxonomists. Not surprisingly, existing collections are not always taxonomically treated and very few recent publications are dedicated to this group.

Our recent studies of Eulimidae in southern Vietnam, Nha Trang Bay (Dgebuadze & Kantor 2010, in press), have doubled the number of species known for Vietnam (Hylleberg & Kilburn 2003; Thach 2005, 2007). A high proportion of eulimid specimens were collected on commatulid crinoids.

Although eulimids are believed to be parasitic (including both ecto- and endoparasites), only few of them were found in association with host organisms. Bouchet & Warén (1986) suggested that many eulimids do not live in permanent association with their host organisms and spend most of their lifetime as free-living, visiting individuals of host species occasionally.

At present there are five Eulimidae genera that are known to inhabit crinoids (Warén 1983). Species of *Tropiometricola* Warén, 1981 form galls on their

hosts and have a globose shell. *Crinolamia* Bouchet & Warén, 1979 species are characterized by the vitreous shell with evenly-rounded whorls and were found on stalked crinoids from the Norwegian Sea. Species of *Curveulima* Laseyron, 1955 are parasites of comatulids and have a small, more or less conical, vitreous and transparent shell, with rounded obtuse apex (Laseyron 1955). *Goodingia* Lützen, 1972 species have an oval, transparent, smooth shell, with numerous faint growth lines and a few extremely distinct ones at irregular intervals and were found in comatulids (Lützen 1972; Dgebuadze & Kantor 2010).

Finally, representatives of the genus *Annulobalcis* Habe, 1965 have a more or less slender shell with moderately high aperture with a strongly projecting outer lip, and usually brightly coloured body (Habe 1965; Warén 1983). The operculum is elongated, oval in shape, thin, light yellow. Most species of this genus were found in association with comatulids. They do not form galls, and are attached to their hosts using well-developed proboscis. Pseudopallium is absent. At present this genus includes six described species which inhabit Indo-West Pacific and Brazil waters (Habe 1965, 1974; Simone & Martins 1995; Simone 2002).

Most common species of Eulimidae associated with crinoids in Nha Trang Bay can be assigned to the genus *Annulobalcis* due to their shell morphology and bright characteristic colouration of the soft body in some of them. Provisionally they were treated as two separate species (both were still undescribed), however further studies have shown that both provisional species are not uniform morphologically and that variations within each "species" are correlated with host specificity. This suggests that each provisional species may in fact comprise a species complex. Present study aimed at morphologic and molecular analysis of *Annulobalcis* spp. associated with crinoids from Nha Trang Bay in order to reveal taxonomic structure of this grouping.

MATERIAL AND METHODS

MATERIAL COLLECTION

Material for present study was collected in spring and summer of 2008-2010 in Nha Trang Bay

(South China Sea) near the islands Tre, Mun and Nok (for exact coordinates refer to species descriptions). Host specimens of crinoids were collected manually at depths from 0 to 15 m using SCUBA equipment. Immediately after picking up, each host individual has been placed in zip-lock bag to avoid loss of symbionts. On the boat, specimens were washed in a 1-3% ethyl alcohol solution and examined visually to remove all associated symbionts. Hosts and symbiont specimens were fixed in ethyl alcohol for further studies, some in 96% ethanol for subsequent sequencing. In laboratory, hosts and symbionts were identified, measured and photographed. Photographs of live specimens were taken under water or in aquaria.

DNA EXTRACTION AND PCR

14 specimens of *Annulobalcis* spp. were prepared for molecular studies. These specimens were photographed and soft bodies were extracted from the shells as delicately as possible. For DNA isolation we used routinely Diatom Prep 100 kit, following the manufacturer's standard protocol. Problematic specimens were treated with Spin-Columns.

10 ng of genomic DNA was used as a template for polymerase chain reaction (PCR) with standard mitochondrial Cox1 primers (Folmer *et al.* 1994): LCO1490 5'-GGTCAACAAATCATAAAGA TATTGG-3'H-8229

CO2198 5'-TAAACTTCAGGGTGACCAA AAAATCA-3'-8505

PCR were set up in total volume of 20 µl. The PCR cycling profiles were as follows: initial denaturation (94°C, 3 min); followed first by five cycles of denaturation (94°C, 90 sec), annealing (45°C, 90 sec) and extension (72°C, 90 sec) and then by 35 cycles of denaturation (94°C, 90 sec), annealing (51°C, 90 sec) and extension (76°C, 60 sec). The resulting PCR products were purified by direct purification from the PCR mixture and prepared for sequencing.

PHYLOGENETIC ANALYSIS

Mit-Cox1 sequences for three other species of the family Eulimidae and one species of Cerithiopsidae (Table 1) were used as outgroups for phylogenetic

TABLE 1. — GenBank accession numbers for Cox1 sequences used in the analysis.

Species	GenBank accession number
<i>Balcis eburnea</i> (A. Adams, 1861)	AF120636.1
<i>Stilifer</i> sp.	DQ916507.1
<i>Thyca crystallina</i> (Gould, 1846) 1	FJ386371.1
<i>T. crystallina</i> 2	FJ386370.1
<i>T. crystallina</i> 3	FJ386369.1
<i>T. crystallina</i> 4	FJ386368.1
<i>T. crystallina</i> 5	FJ386367.1
<i>Ataxocerithium</i> sp. (Cerithiopsidae)	AY296835.1
<i>Annulobalcis albus</i> n. sp.	JF717844-JF717846
<i>Annulobalcis wareni</i> n. sp.	JF717841-JF717843
<i>Annulobalcis vinarius</i> n. sp.	JF717850-JF717854
<i>A. maculatus</i> n. sp. paratype 1	JF717847
<i>A. maculatus</i> n. sp. paratype 2	JF717848
<i>A. maculatus</i> n. sp. paratype 3	JF717849

analyses. Cox1 sequences were automatically aligned using Clustal W2 (Larkin *et al.* 2007) multiple alignment algorithm accessible online at: <http://www.ebi.ac.uk/Tools/msa/clustalw2/> and have been modified manually with BioEdit 7.0.5.2 software.

The best fitting model of nucleotide substitution was defined with Modeltest (Posada & Crandall 2001). For Bayesian analysis, the general time-reversible model with invariable sites and a gamma-distributed rate heterogeneity parameter (GTR+G+I) was selected. Phylogenetic reconstructions were conducted by Bayesian analysis, consisting of two Markov chains, 100 000 generations each, run in six parallel analyses in MrBayes (Ronquist & Huelsenbeck 2003). The sampling frequency was set up as one tree each 100 generations. When log-likelihood scores were found to stabilize, consensus trees were calculated after omitting first 10% of trees. Bayesian posterior probabilities were calculated as branch support values.

ABBREVIATIONS

AH	aperture height;
AW	aperture width;
LWH	last whorl height;
MNHN	Muséum national d'Histoire naturelle, Paris;
PP	posterior probability;
SH	shell height;
SW	shell width;
ZIN	Zoological Institute of Russian Academy of Sciences, St. Petersburg.

TABLE 2. — Parameters of the nucleotide substitution model.

Marker	Model	Base frequencies	Substitution rates	I	G
Cox1	TVM+I+G	$\pi A = 0.2784$ $\pi C = 0.1689$ $\pi G = 0.1575$ $\pi T = 0.3951$	$[A-C] = 0.0000$ $[A-G] = 19.8214$ $[A-T] = 0.4393$ $[C-G] = 3.9613$ $[C-T] = 19.8214$ $[G-T] = 1.0000$	0.545	0.898

RESULTS

MOLECULAR PHYLOGENY

After mitochondrial Cox1 sequences were aligned, the molecular dataset of 534 positions has been obtained. Of these, 310 nucleotide positions were found to be constant and 194 positions gave parsimony informative characters. TVM+I+G has been chosen as a best fitting model of nucleotide substitution (parameters of the model are given in Table 2).

The consensus tree is characterized by high support of all terminal clades, while deep nodes are not supported. 14 examined specimens of the genus *Annulobalcis* clustered to form four distinct groups (1 to 4) (Fig. 1). All these groups are well supported (PP 0.97 to 1.00) and together form a single clade on a tree, also well supported (PP 1.00). The branching order and supports values in this clade allow judgment on the relationships between four groups.

Groups 3 and 4 are composed of forms with uncoloured bodies and grouping together (although not with significant support). Contrary, two similar looking groups (1 and 2) that are characterized by brightly coloured red body tissues with white spots are distant from each other. Group 2 is in the same clade with uncoloured groups 3 and 4, while group 1 is a sister one to the clade formed by groups 2-4 (Fig. 1).

All specimens were collected sympatrically (sometimes syntopically, on the same host specimen). Each group under consideration is reciprocally monophyletic and characterized by distinct morphological peculiarities. All this suggests that these four entities certainly correspond to different species.

Low support of basal nodes did not allow to resolve monophyly of Eulimidae in general. *Balcis eburnea* (A. Adams, 1861), *Stilifer* sp. and *Thyca crystallina* (Gould, 1846) formed a distinct clade, sister to *Annulobalcis* clade and to *Ataxocerithium* sp. as well, due to lack of resolution in basal nodes of the consensus tree.

Below are descriptions of the new species.

SYSTEMATICS

Family EULIMIDAE Philippi, 1853

Genus *Annulobalcis* Habe, 1965

TYPE SPECIES. — *Annulobalcis shimazui* Habe, 1965 (by monotypy).

Annulobalcis albus n. sp.
(Figs 2; 6A, B)

Annulobalcis sp. 1 – Dgebuadze & Kantor 2010 (*partim*): figs 2a, v; 4a.

TYPE MATERIAL. — Vietnam. South China Sea, Nha Trang Bay, Mun Island (Rock Rom), 12°10'12.51"N, 109°18'46.06"E, 5-7 m, holotype (MNHN 24190). Same data as holotype, 5 paratypes (ZIN 61118-61120, MNHN 24191).

OTHER MATERIAL EXAMINED. — 450 specimens from crinoids *Oxycomanthus benetti* (Müller, 1841) (Comasteridae), 3 specimens sequenced (JF717844-JF717846), stored in the Laboratory of Marine Invertebrates of A. N. Severtzov Institute of Ecology and Evolution.

DISTRIBUTION. — South China Sea, Nha Trang Bay.

ETYMOLOGY. — From the Latin “albus”, white reflecting colouration of soft parts.

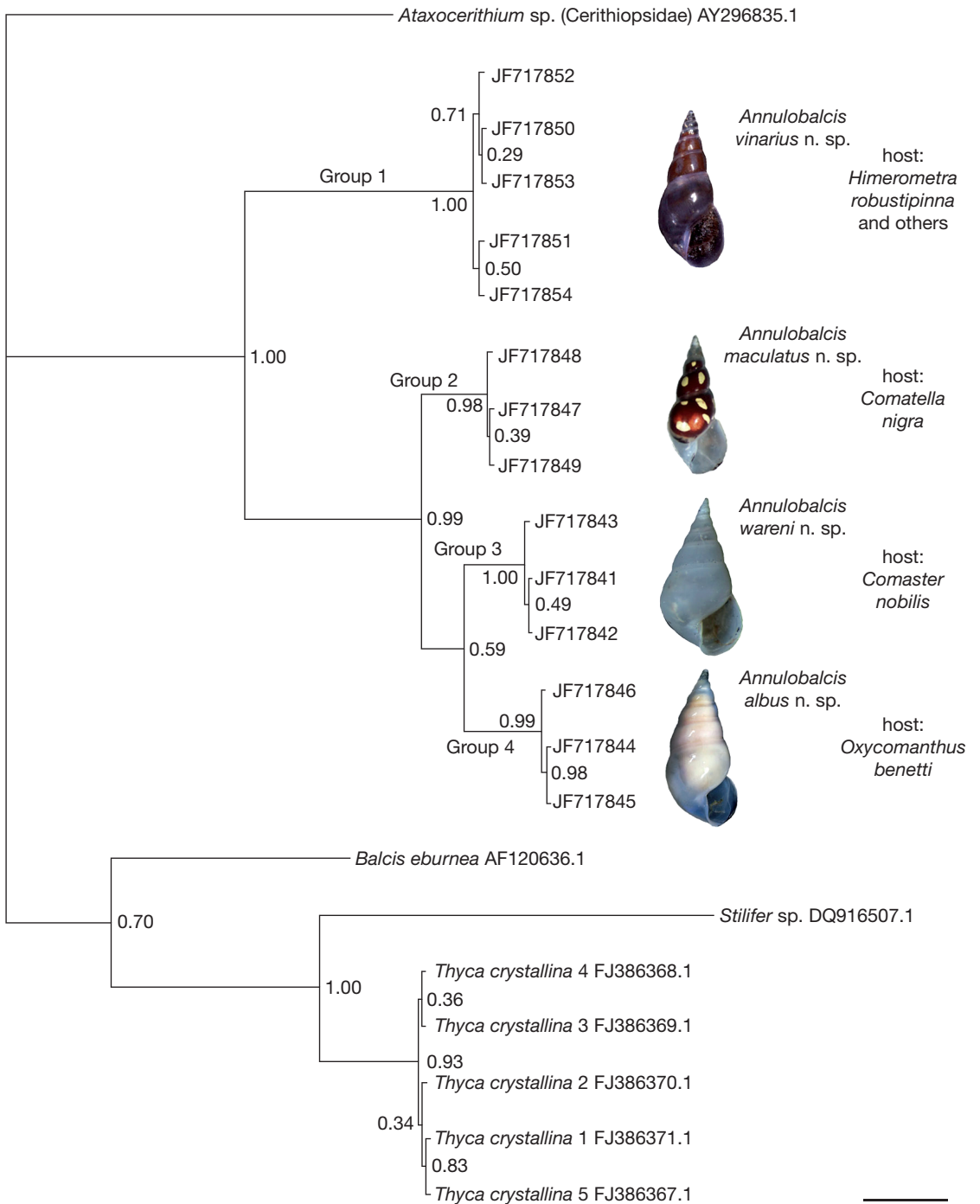


FIG. 1. — Consensus tree obtained for mitochondrial Cox1 sequences, using Bayesian analysis. Posterior probabilities are indicated for each node. Scale bar: 0.1.

TABLE 3. — Measurements (in mm) of holotype, paratypes and additional specimens of *Annulobalcis albus* n. sp. Remark: specimens 1-3 are from the same host as the sequenced ones.

Specimen	SH	SW	SW/SH	AH	AH/SH	AW	LWH	LWH/SH
Holotype (MNHN 24190)	6.0	2.5	0.42	2.3	0.38	1.2	3.6	0.60
Paratype 1 (ZIN 61118)	3.1	1.7	0.55	1.1	0.35	0.8	2.4	0.77
Paratype 2 (ZIN 61119)	3.9	1.9	0.49	1.7	0.43	0.9	2.7	0.69
Paratype 3 (ZIN 61120)	4.9	2.0	0.41	2.0	0.41	1.0	2.8	0.57
Paratype 4 (MNHN 24191)	5.0	2.1	0.42	1.9	0.38	1.0	3.0	0.60
Paratype 5 (MNHN 24191)	5.2	2.3	0.44	2.2	0.42	1.1	2.9	0.56
Specimen 1	4.8	2.0	0.42	1.9	0.39	1.1	3.0	0.62
Specimen 2	4.2	2.0	0.48	1.9	0.45	1.1	2.8	0.67
Specimen 3	3.7	1.5	0.40	1.5	0.45	0.8	2.5	0.67

DESCRIPTION (HOLOTYPE)

Shell narrow, elongated-conical, glossy, translucent porcellaneous, smooth except numerous frequently irregularly spaced axial lines. Shell of nine whorls.

Larval shell pointed, with small nucleus, smooth, border with teleoconch not pronounced. Four upper shell whorls with lower expansion rate than other whorls of teleoconch. Axis slightly curved. Suture shallow, false suture (for definition see Bouchet & Warén 1986) seen by transparency. Teleoconch whorls slightly convex, last whorl high, comprising 0.6 of shell height.

Aperture broadly ovate, with angulated junction of columellar and parietal margins, 0.38 of shell height. Columellar margin short, weakly convex, about half aperture height. Parietal margin evenly rounded without callus. Outer lip in ventral view evenly rounded, in lateral view forms deep anal sinus near suture, lip most protruded in its middle part.

Soft parts hardly visible through the shell.

Measurements

See Table 3.

Host

Molluscs were found on cirri of crinoids *Oxycomanthus benetti* (Comasteridae).

Colouration

In live specimens body translucent (Fig. 6A, B). Foot translucent with longitudinal stripe of lemon-yellow colour. Tentacles transparent with longitudinal lemon yellow stripes, extending

for entire tentacles' length. Eyes well developed, black, situated at tentacles base. Proboscis present. Pseudopallium absent. Operculum present, oval, spiral, nucleus close to columellar margin (Fig. 3N).

Intraspecific variability

Paratypes are very similar to holotype in shell shape and morphology. Shell tending to become more slender (decreasing of SW/SH ratio) with increasing of its height. There is also a weak tendency of decreasing of relative height of the last whorl with increasing of the shell height.

Due to their small size and fragile shell the sequenced specimens were destroyed. The remaining specimens collected from the same host specimen (Fig. 2K, L; Table 3, specimens 1-3) are very similar to the type series and no doubt of their conspecificity remains.

REMARKS

Most species of the genus *Annulobalcis* are characterized by more or less pronounced shell sculpture (A. *yamamotoi* Habe, 1974, A. *shimazui* Habe, 1965, A. *aurisflamma* Simone & Martins, 1995, A. *procera* Simone, 2002), usually visible under light microscope or even by naked eye. The presence of the spiral sculpture was considered as characteristic of the genus. Thus our new species is markedly different from typical representatives of *Annulobalcis*. Nevertheless Warén (1981) described *Annulobalcis marshalli* Warén, 1981 from deep waters of New Zealand (on the crinoid *Crotalometra rustica* Clark, 1909) also lacking spiral sculpture.

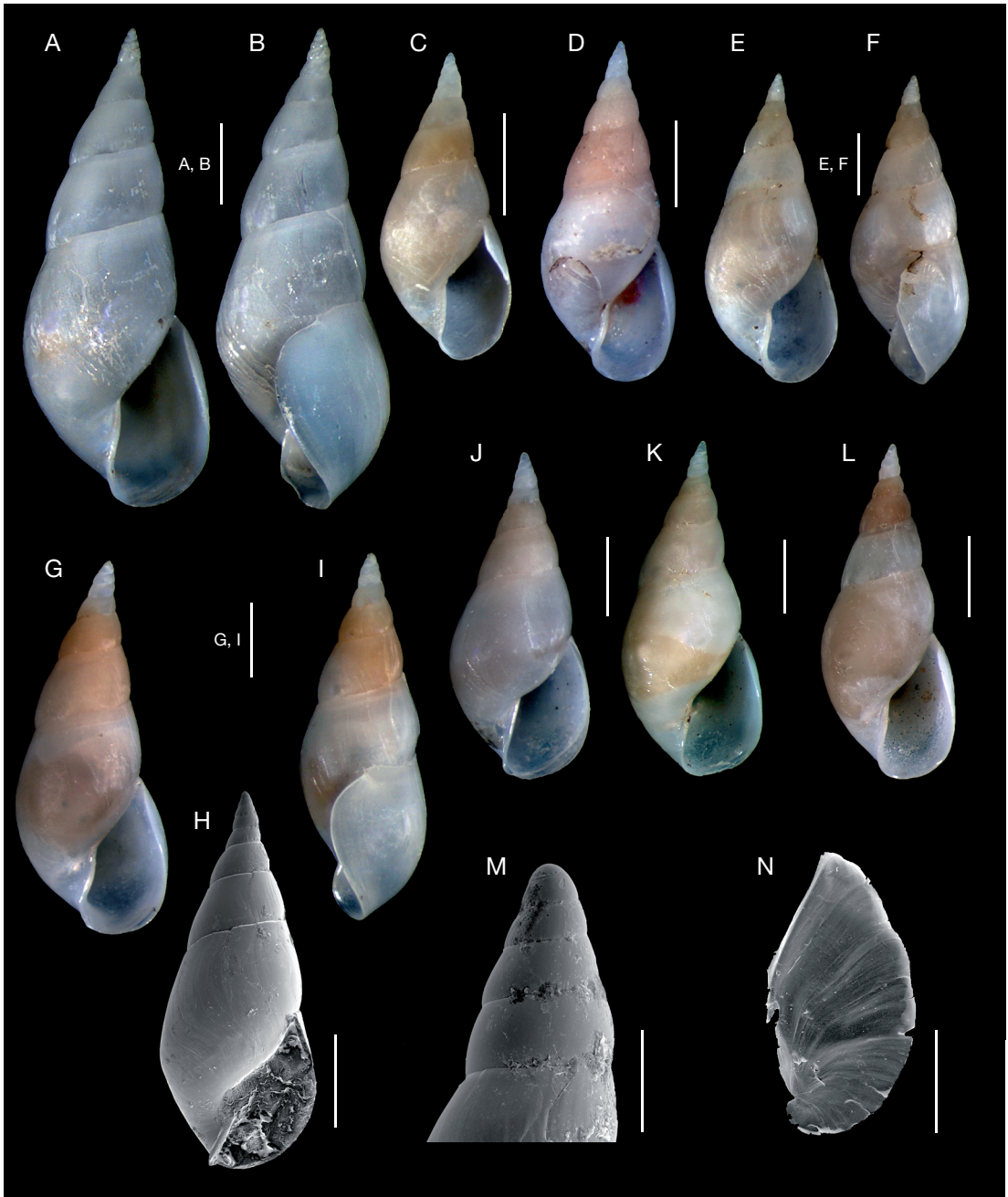


FIG. 2. — *Annulobalcis albus* n. sp.: **A, B**, holotype (MNHN 24190); **C**, paratype 1 (ZIN 61118); **D**, paratype 2 (ZIN 61119); **E, F**, paratype 3 (ZIN 61120); **G, I**, paratype 4 (MNHN 24191); **H**, specimen 3 (Table 3) collected from the same host as the sequenced ones, SEM of the shell; **J**, paratype 5 (MNHN 24191); **K, L**, specimens 1 and 2 (Table 3) collected from the same host as the sequenced ones; **M**, specimen 3 apex, SEM; **N**, same, operculum, SEM. Scale bars: A-L, 1 mm; M, 200 μ m; N, 500 μ m.

TABLE 4. — Measurements (in mm) of holotype, paratypes and additional specimens of *Annulobalcis wareni* n. sp. Remark: specimens 1-2 are from the same host as the sequenced ones.

Specimen	SH	SW	SW/SH	AH	AH/SH	AW	LWH	LWH/SH
Holotype (MNHN 24192)	3.8	1.9	0.50	1.3	0.34	0.9	2.3	0.60
Paratype 1 (ZIN 61121)	5.4	2.5	0.46	2.0	0.37	1.1	3.0	0.55
Paratype 2 (MNHN 24193)	5.6	2.0	0.36	1.9	0.34	1.2	2.9	0.52
Specimen 1	2.3	1.2	0.52	0.8	0.38	0.6	1.4	0.61
Specimen 2	3.0	1.5	0.50	1.2	0.4	0.7	1.9	0.63

Warén also transferred another species, *Apicalia cicatricosa* Warén, 1981 (from unknown host) with very indistinct and scattered spiral sculpture to *Annulobalcis*. Thus the absence of the sculpture does not preclude including *Annulobalcis albus* n. sp. in the genus.

The present species resembles *Annulobalcis marshalli* in smooth shell, but differs by relatively broader shell with taller last whorl and aperture. Also the new species is significantly, more than twice smaller.

In shells proportions the new species is similar to *Annulobalcis cicatricosa* (Warén, 1981) (Eagle Island, Queensland, Australia), which has a 0.43 SW/SH ratio in holotype, but has slower increasing and more numerous as well as less convex teleoconch whorls. For comparison with *A. wareni* n. sp. see the corresponding species.

Annulobalcis wareni n. sp.
(Figs 3; 6F)

TYPE MATERIAL. — Vietnam. South China Sea. Nha Trang Bay, Mun Island (Rock Rom), 12°10'12.51"N, 109°18'46.06"E, 5-7 m, holotype (MNHN 24192). Same data as holotype, 2 paratypes (ZIN 61121, MNHN 24193).

OTHER MATERIAL EXAMINED. — 12 specimens from *Comaster nobilis* (Carpenter, 1884) (Comasteridae), 3 specimens sequenced (JF717841-JF717843), stored in the Laboratory of Marine Invertebrates of A. N. Severtzov Institute of Ecology and Evolution.

DISTRIBUTION. — South China Sea, Nha Trang Bay, Vietnam.

ETYMOLOGY. — The present species is named in honour of Dr Anders Warén, the leading specialist in family Eulimidae as well as in other molluscs.

DESCRIPTION (HOLOTYPE)

Shell conical, glossy, greyish white, semi transparent, smooth except thin sigmoid scars corresponding to outer lip form.

Larval shell is pointed, conical, smooth, consists of > 4 slightly convex whorls, diameter 400 µm. Boundary between protoconch and teleoconch marked by thin, but distinct sigmoid scar. Teleoconch of four whorls.

Larval shell with axis curved and expansion rate lower than other whorls of teleoconch. Suture shallow, false suture seen by transparency. Teleoconch whorls slightly convex. Last whorl comprises 0.6 of shell height.

Aperture ovate with angulated junction of columellar and parietal margins, 0.34 of shell height. Columellar margin weakly convex, more than half of aperture height. Parietal margin evenly rounded, without callus. Outer lip evenly rounded in ventral view, in lateral view forms deep anal sinus near suture, lip most protruded in its middle part.

Soft parts are visible through shell in paratypes.

Measurements

See Table 4.

Host

Molluscs were found on cirri and lower side of calyx of crinoids *Comaster nobilis* (Comasteridae).

Morphology and colouration

Body colourless in live specimens (Fig. 6F). Foot semi-transparent with central longitudinal non transparent white stripe. Tentacles yellow. Eyes well developed, black, situated at tentacles base. Proboscis present, pseudopallium absent. Operculum present (Fig. 3G), spiral, oval, nucleus close to columellar margin.

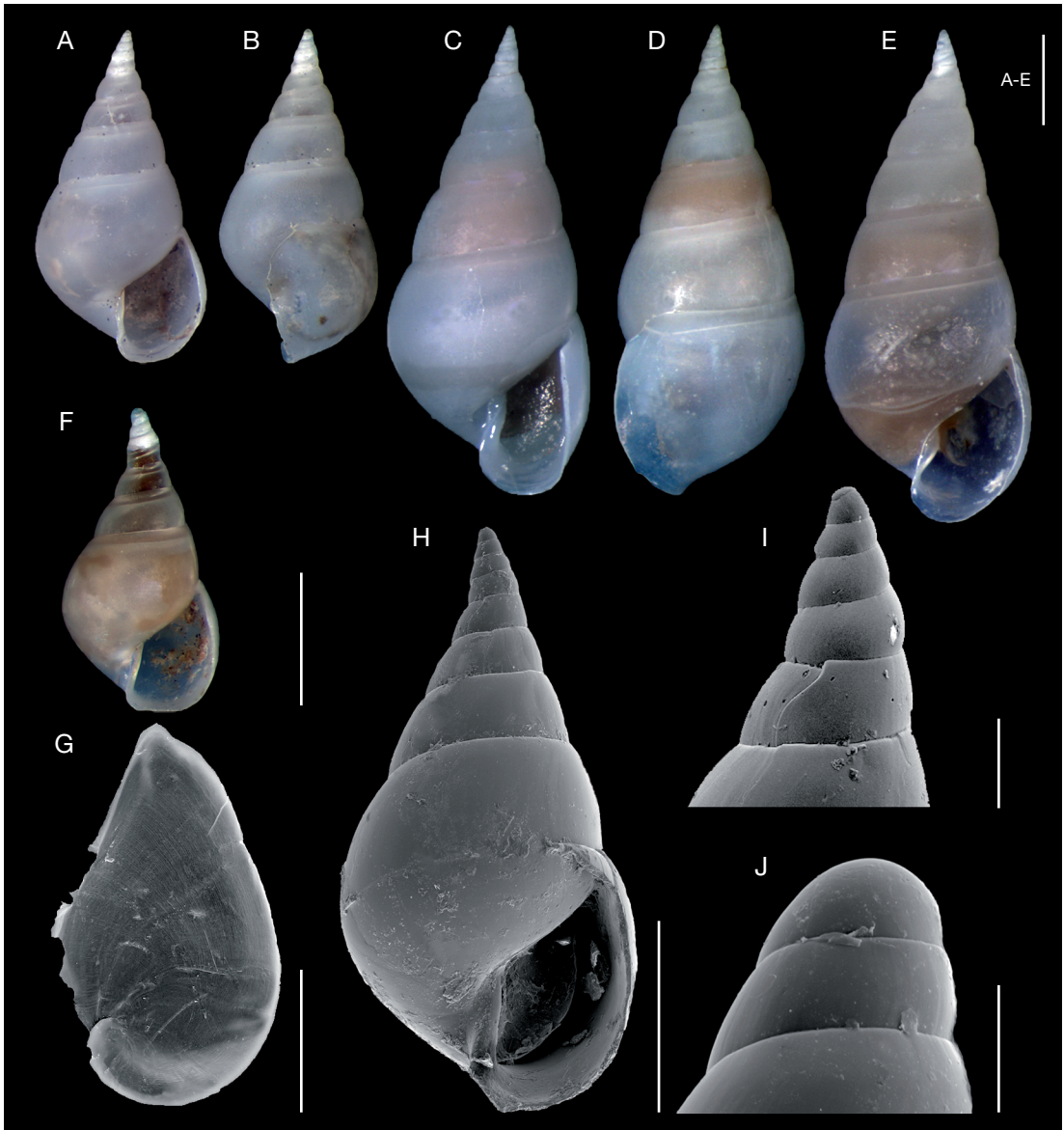


FIG. 3. — *Annulobalcis wareni* n. sp.: **A, B**, holotype (MNHN 24192); **C, D**, paratype 1 (ZIN 61121); **E**, paratype 2 (MNHN 24193); **F**, specimen 1 (Table 5) collected from the same host as the sequenced ones; **G-J**, specimen 2 (Table 5) collected from the same host as the sequenced ones; **G**, operculum, SEM; **H**, SEM of the shell; **I, J**, apex in different views, SEM. Scale bars: A-F, H, 1 mm; G, 500 μ m; I, 200 μ m; J, 100 μ m.

Intraspecific variability

Paratypes are very similar to holotype in shell shape and morphology. Shell tending to become more slender (decreasing of SW/SH ratio) with increasing

of its height. There is also a tendency to decreasing of LWH/SH ratio with increasing of the shell height. Paratype 1 has more extended and protruded outer lip than holotype.

TABLE 5. — Measurements (in mm) of holotype, paratypes and additional specimens of *Annulobalcis vinarius* n. sp. Remarks: specimen 1 was sequenced; specimens 2-3 are from the same host as the sequenced ones.

Specimen	SH	SW	SW/SH	AH	AH/SH	AW	LWH	LWH/SH
Holotype (MNHN 24194)	2.9	1.2	0.41	1.1	0.38	0.6	1.7	0.59
Paratype 1 (ZIN 61122)	2.0	1.0	0.50	0.9	0.45	0.5	1.3	0.65
Paratype 2 (ZIN 61123)	2.9	1.2	0.41	1.2	0.41	0.7	1.7	0.59
Paratype 3 (MNHN 24195)	3.5	1.5	0.43	1.4	0.40	0.9	2.2	0.63
Paratype 4 (MNHN 24195)	7.0	2.8	0.40	2.4	0.34	1.4	4.0	0.57
Specimen 1	7.3	2.8	0.38	2.1	0.29	1.3	3.9	0.53
Specimen 2	4.7	2.1	0.45	1.9	0.40	1.2	2.9	0.62
Specimen 3	2.3	0.9	0.39	0.9	0.39	0.8	1.4	0.60

Due to their small size and fragile shell the sequenced specimens were destroyed. The remaining specimen collected from the same host specimen (Fig. 3F; Table 4, specimen 1) is very similar to the type series and no doubt of conspecificity of sequenced specimens and type material remains.

REMARKS

The new species is different from *Annulobalcis shimazui* in absence of spiral sculpture and thus resembles *Annulobalcis marshalli*, but differs from this species by shell form, size and lower AH/SH ratio. The present species differs from *Annulobalcis cicatricosa* by irregular growth of the shell and less convex teleoconch whorls. The new species resembles *Annulobalcis albus* n. sp. in shell shape. Younger specimens are characterized by much more swollen and globose last whorl than in *A. albus* n. sp. Nevertheless the adults are more similar in shell proportions, although *A. wareni* n. sp. has in general more convex last whorl, obtusely angled. Both species are distinctly different in Cox1 sequence and live on different host species.

Annulobalcis vinarius n. sp. (Figs 4; 6C-E)

Annulobalcis sp. 2 – Dgebuadze & Kantor 2010: figs 2b, g; 4b.

TYPE MATERIAL. — Vietnam. South China Sea, Nha Trang Bay, Mun Island (Rock Rom), 12°10'12.51"N, 109°18'46.06"E, 5-7 m, holotype (MNHN 24194). Same data as holotype, 4 paratypes (ZIN 61122, 61123, MNHN 24195).

OTHER MATERIAL EXAMINED. — 1440 specimens from several species of crinoids, 5 specimens sequenced (JF717850-JF717854), stored in the Laboratory of Marine Invertebrates of A. N. Severtzov Institute of Ecology and Evolution.

DISTRIBUTION. — South China Sea, Nha Trang Bay.

ETYMOLOGY. — From the Latin "vinarius", wine merchant, reflecting wine-red colours of soft parts of live specimens.

DESCRIPTION (HOLOTYPE)

Shell thin, transparent, narrow, glossy, elongated-conical, with well-developed irregularly situated growth lines and scars. Shell of 8.5 whorls.

Larval shell of about two smooth broad whorls, separated by shallow impressed suture. Boundary between protoconch and teleoconch marked by changing of suture type and appearance of false suture seen by transparency (not visible on SEM photograph; Fig. 4I).

Teleoconch whorls slightly convex. Expansion rate nearly constant. Spiral sculpture not pronounced. Suture clearly adpressed. Last whorl high, 0.59 of shell height. Shell axis not curved.

Aperture broadly ovate, with visible angle between columellar and parietal margins, 0.38 of shell height. Parietal margin evenly rounded, slightly convex, without callus. Columellar margin short, slightly convex, nearly flat, about $\frac{2}{3}$ of aperture height. Outer lip in ventral view evenly rounded, in lateral with distinct sinus below suture, evenly protruded and curved in middle part.

Soft parts clearly visible through shell.

Measurements
See Table 5.

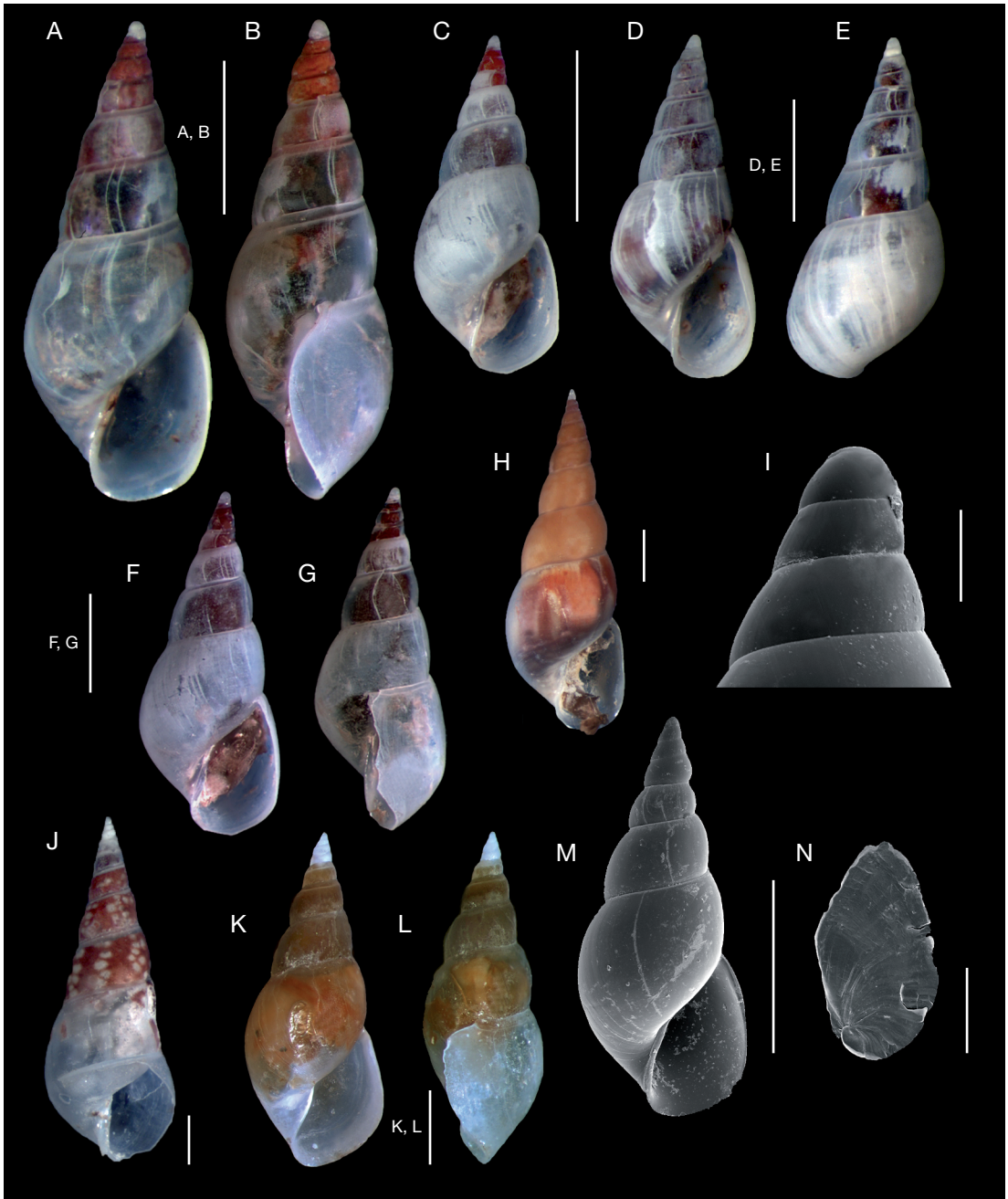


FIG. 4. — *Annulobalcis vinarius* n. sp.: **A, B**, holotype (MNHN 24194); **C**, paratype 1 (ZIN 61122); **D, E**, paratype 2 (ZIN 61123); **F, G**, paratype 3 (MNHN 24195); **H**, paratype 4 (MNHN 24195); **I**, specimen 3 (Table 5) collected from the same host as the sequenced ones, apex, SEM; **J**, sequenced specimen 2 (Table 5) (GenBank no. JF717854); **K, L**, specimen, collected from the same host as the sequenced ones; **M**, specimen 3, SEM of the shell; **N**, same, operculum, SEM. Scale bars: A-H, J-M, 1 mm; I, 100 µm; N, 500 µm.

Host

Holotype and paratypes were found on calyx (lower and upper side), on arms, on cirri of crinoids *Himerometra robustipinna* Carpenter, 1881 (Himerometridae). Other specimens were found on a wide range of hosts: *Comatella nigra* Carpenter, 1888 (Comasteridae); *Stephanometra indica* (Smith, 1876); *Dichrometra flagellata* (Müller, 1841) (Mariamtridae); *Stephanometra tenuipinna* (Hartlaub, 1890); *Amphimetra ensifera* (A. H. Clark, 1908) (Himerometridae). On a specimen of *Comatella nigra* it was co-occurring with *A. maculatus* n. sp.

Morphology and colouration

In live specimens body dark red with small white spots arranged in axial bands regularly situated on whorls of visceral mass. On last whorl, spots irregularly scattered. Foot large, narrow, dark red with white spots and white edges. Tentacles red with white tips. Eyes well developed, black, situated at base of tentacles. Proboscis present. Mantle transparent with well-marked white edge. Pseudopallium absent. Operculum present, oval, spiral, nucleus close to columellar margin (Fig. 4N).

Intraspecific variability

Paratypes are very similar to holotype in shell shape and morphology with slight variation in slenderness and relative aperture height. The smallest paratype has a broader shell, but the tendency is not well pronounced. The largest paratype has more or less regularly distributed white spots on the last body whorl.

REMARKS

The present species resembles *Annulobalcis yamamotoi* from Japan and *Annulobalcis aurisflamma* from Brazil in transparent shell and brightly coloured spotted soft parts, but differs in the absence of spiral sculpture, as well as in the shell shape, shell width and height ratio. For the differences with *A. maculatus* n. sp. see the description of the latter.

One of the specimens deposited four egg-capsules during observations (Fig. 6E). Egg capsules are attached to the host, bean-shaped, semi-transparent up to 5.5 mm in length. Eggs are very numerous and small.

Annulobalcis maculatus n. sp.

(Fig. 5)

TYPE MATERIAL. — Vietnam. South China Sea, Nha Trang Bay, Mun Island (Rock Rom), 12°10'12.51"N; 109°18'46.06"E, 5-7 m, holotype (MNHN 24196). Same data as holotype, 3 paratypes sequenced (ZIN 61124, JF717847; ZIN 61125, JF717848; MNHN 24197, JF717849).

OTHER MATERIAL EXAMINED. — 27 specimens from crinoids *Comatella nigra* (Comasteridae), stored in the Laboratory of Marine Invertebrates of A. N. Severtzov Institute of Ecology and Evolution.

DISTRIBUTION. — South China Sea, Nha Trang Bay.

ETYMOLOGY. — From the Latin "maculatus", spotty, shows the colouration of soft parts in live specimens, red with big light spots.

DESCRIPTION (HOLOTYPE)

Shell thin, transparent, narrow, glossy, elongated-conical, with well-developed irregularly situated growth lines and scars. Shell of > 9 whorls.

Larval shell smooth consists of about > 4 whorls. Boundary between protoconch and teleoconch marked by thin sigmoid scar. Protoconch slightly curved, its whorls less convex and have lower expansion rate than whorls of teleoconch.

Teleoconch of five whorls. Suture shallow, false suture seen by transparency, narrow. Body whorl high, 0.56 of shell height. Aperture ovate with angulated junction of columellar and parietal margins, 0.36 of shell height. Parietal margin rounded, slightly convex, without callus. Columellar margin short, bulging, more than half of aperture height, slightly deflected out. Outer lip strongly convex in ventral view in middle part, nearly flat in upper part; basal part with rounded angle; in lateral view, sigmoid shape, with marked anal sinus near the suture.

Soft parts clearly visible through shell.

Measurements

See Table 6.

Host

Molluscs were found on lower side of calyx and on cirri of crinoids *Comatella nigra*.

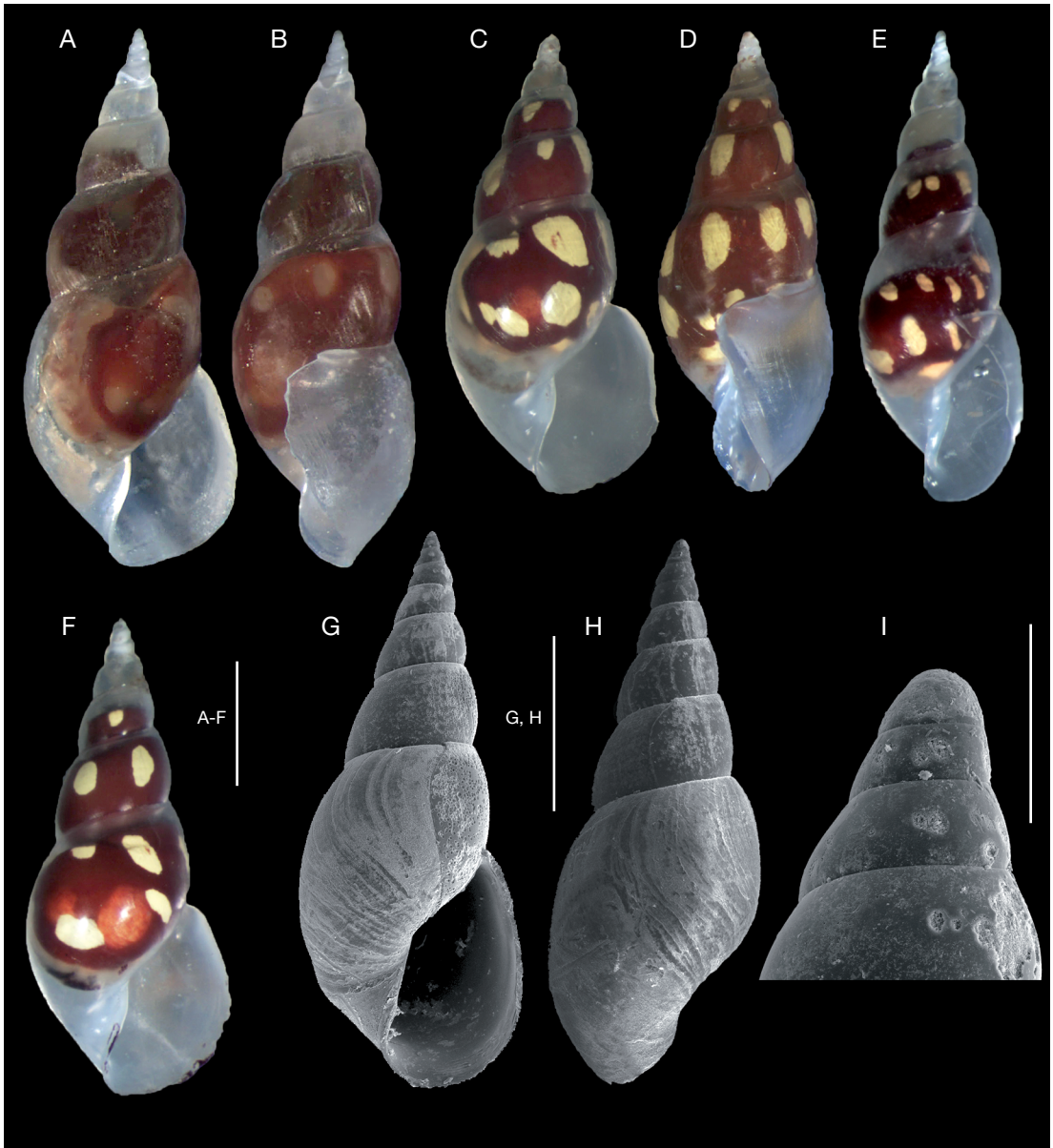


FIG. 5. — *Annulobalcis maculatus* n. sp.: **A, B**, holotype (MNHN 24196); **C, D**, paratype 1 (ZIN 61124); **E**, paratype 2 (ZIN 61125); **F**, paratype 3 (MNHN 24197); **G-I**, specimen from the same host as the types; **G, H**, shell SEM; **I**, apex, SEM. Scale bars: A-H, 1 mm; I, 200 μ m.

Morphology and colouration

In live specimens body dark red with large yellowish spots irregularly situated on the visceral mass

whorls. Eyes well developed, black, situated at base of tentacles. Proboscis present. Operculum present, similar in morphology to other species.

TABLE 6. — Measurements (in mm) of holotype and paratypes of *Annulobalcis maculatus* n. sp.

Specimen	SH	SW	SW/SH	AH	AH/SH	AW	LWH	LWH/SH
Holotype (MNHN 24196)	6.4	2.5	0.39	2.4	0.36	1.4	3.7	0.56
Paratype 1 (ZIN 61124)	5.5	2.5	0.45	2.2	0.4	1.3	3.5	0.64
Paratype 2 (ZIN 61125)	5.6	2.0	0.36	2.1	0.37	1.2	3.4	0.61
Paratype 3 (MNHN 24197)	5.7	2.5	0.44	1.6	0.4	1.6	3.5	0.61

Intraspecific variability

Paratypes are very similar to holotype in shell shape and morphology.

The shell growth parameters change with size: there is a weak tendency of decreasing of SW/SH ratio with increasing of shell height. There is also a tendency of decreasing of LWH/SH ratio with increasing of the shell height.

REMARKS

The present species resembles *Annulobalcis vinarius* n. sp. in shell form, absence of spiral sculpture, host preference and distribution. However it differs by a lower number of whorls, more convex whorls, colouration, form and type of spots distribution on soft parts. Besides both species are clearly separated by mitochondrial Cox1 sequence.

DISCUSSION

One of the results of the current extensive studies of the biodiversity is the reassessment of the share of symbiotic molluscs in total marine biodiversity. Thus in New Caledonia the three most abundant monophyletic families of gastropods comprised symbiotic species (in the order of species number decrease): Triphoridae (associated with sponges), Eulimidae (parasites of echinoderms) and Pyramidellidae (parasitizing other molluscs and other invertebrates) (Bouchet *et al.* 2002).

High proportion of symbiotic and parasitic gastropods suggests that their radiation may be connected with the host specificity. Unfortunately data on the hosts and selectivity of molluscs is rather limited even for Eulimidae, not to mention Pyramidellidae. Most of the species are not permanently attached to their hosts and therefore are found separately.

The genus *Annulobalcis* initially was described for the single species from Sagami Bay, Honshu, *A. shimazui* (Habe 1965). The host was unknown and in the original description it was stated that the species is uncommonly found in the stomach of the starfish *Astropecten* sp. The host for the species is still unknown (Okutani 2000). A second species, *Annulobalcis yamamotoi* (Habe 1974), was described from the crinoid host *Tropiometra afra macrodiscus* (Hara, 1895).

Later additional species were described in or transferred to *Annulobalcis*: *A. cicatricosa*, *A. marshalli* (New Zealand), *A. aurisflamma* and *A. procera* (Brazil). *Annulobalcis marshalli* and *A. aurisflamma* were also described from crinoids, but for remaining species hosts are unknown.

Thus until present, six species of the genus were described (for three of them, crinoids were found to be the hosts), although four still unnamed species (all from crinoids) were mentioned and illustrated in Poppe (2008). Although crinoids appeared to be the only known host organisms for *Annulobalcis* spp., the problem of host specificity was never addressed before.

Our study is the first one using molecular data to identify species of symbiotic eulimid gastropods and deals with several sympatric species of the genus and every specimen (out of nearly 2000) was collected in association with the host.

Three out of the four species described here were found to be strictly species specific in their choice of hosts. The only species that was not strictly host specific, *A. vinarius*, demonstrated clear preference, being mostly found on *Himerometra robustipinna*, while its records on other host species were isolated instances.

Studies of Eulimidae in Nha Trang Bay were a part of the broader survey of symbionts of different Echinodermata, conducted in the Laboratory of

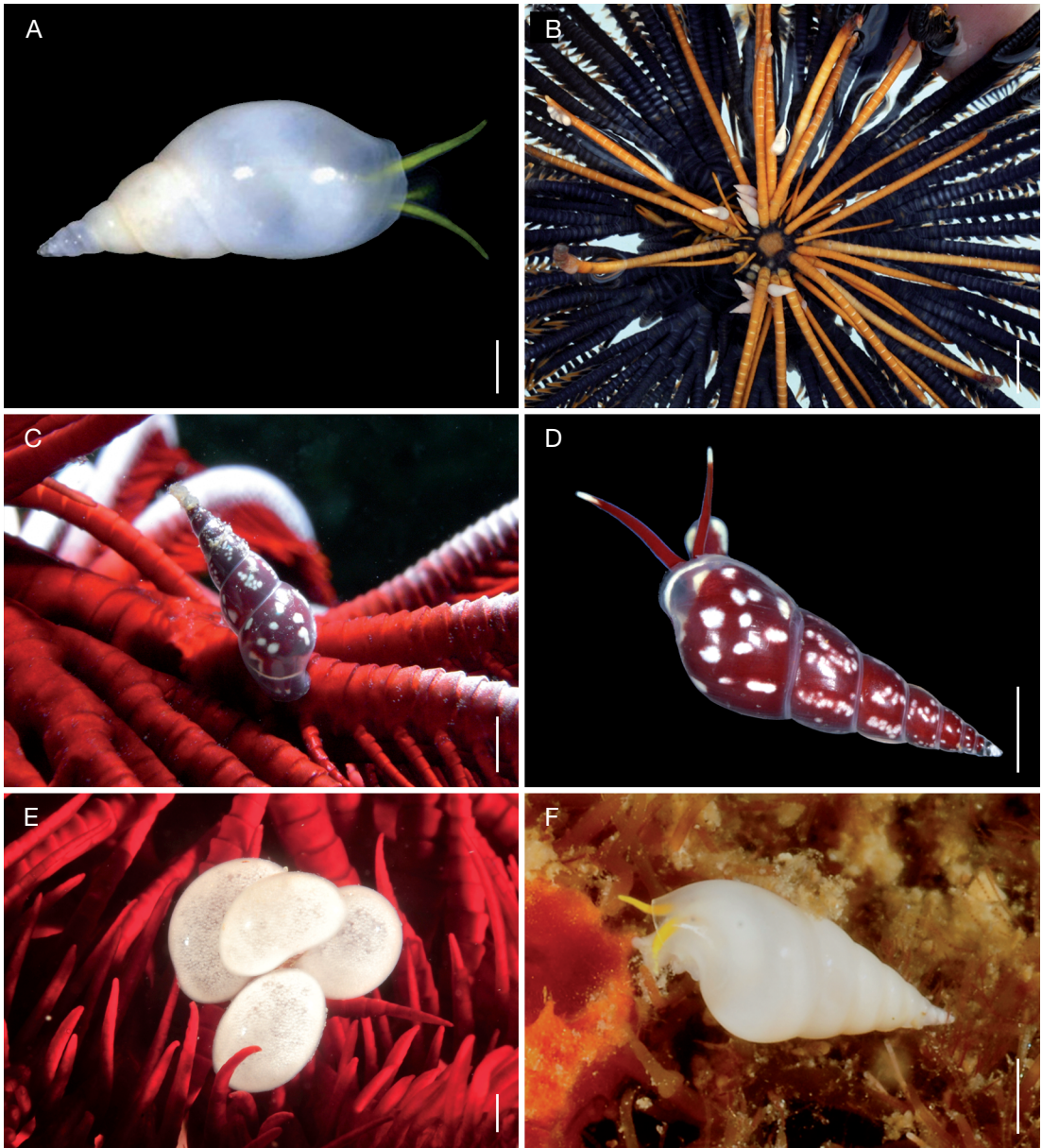


FIG. 6. — **A**, live specimen of *Annulobalcis albus* n. sp.; **B**, *Annulobalcis albus* n. sp. specimens on their host *Oxycomanthus benetti* (Müller, 1841); **C**, *Annulobalcis vinarius* n. sp. on arm of crinoid *Himerometra robustipinna* Carpenter, 1881; **D**, live specimen of *Annulobalcis vinarius* n. sp.; **E**, eggs capsules of *Annulobalcis vinarius* n. sp. on crinoid *Himerometra robustipinna*; **F**, live specimen of *Annulobalcis wareni* n. sp. Scale bars: A, C-F, 1 mm; B, 10 mm.

marine invertebrates of A. N. Severtzov Institute. Over numerous years, 33 species of Crinoidea were found in the bay (Britayev & Mehova 2011) and

only 11 were infected by eulimids. Besides *Annulobalcis*, two species of *Curveulima* Laseron, 1955 and *Goodingia varicosa* (Schepman & Nierstrasz,

1909) were found on different species of crinoids (Dgebuadze & Kantor 2010). There was no case of co-occurrence of any *Annulobalcis* with other species of Eulimidae on the same specimen of the host. Similarly, except a single instance of *A. vinarius* n. sp. and *A. maculatus* n. sp. found on the same host specimen, two *Annulobalcis* species have never been found on the same host.

Thus our results on host specificity are not a collecting artifact but reflect the actual situation with distribution of symbiotic molluscs.

It should be noted that three species most closely related, according to our results, namely *A. albus* n. sp., *A. wareni* n. sp., and *A. maculatus* n. sp. were all found on Crinoidea of the same family, Comasteridae (which is the richest in terms of species number among all the families of crinoids in Vietnam).

Our data demonstrate the high host specificity of *Annulobalcis* spp. at species level and this can explain the co-occurrence of numerous species within a limited area.

The possibility of speciation of parasitic molluscs on different hosts suggests that besides strictly specific species (in our case *A. albus* n. sp., *A. wareni* n. sp. and *A. maculatus* n. sp.) at least some species should be able to colonize other species of hosts. In our case we were able to demonstrate that *A. vinarius* n. sp. fills this requirement and can parasitize several species (although it prefers one). Potentially, species like *A. vinarius* n. sp. could give rise to other host-specific species.

We suggest that in future research the host preference should be taken into account for taxonomical decisions and recognition of similar species of Eulimidae, a group that is poor in shell characters.

Another important character, which we found useful in species discrimination is the colouration of the soft body. Two of our bright-coloured species, *A. maculatus* n. sp. and *A. vinarius* n. sp. can be easily distinguished by the colour pattern. They also can be recognized from other described species of *Annulobalcis* with similarly colourful soft body, *A. shimazui*, *A. yamamotoi*, and *A. aurisflamma*. Therefore we suggest that live specimen's colouration should be taken into account and specimens should be photographed alive prior to fixation.

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