Association between shell morphology of micro-land snails (genus *Plectostoma*) and their predator's predatory behaviour

Predator-prey interactions are among the main ecological interactions that shape the diversity of biological form. In many cases, the evolution of the mollusc shell form is presumably driven by predation. However, the adaptive significance of several uncommon, yet striking, shell traits of land snails are still poorly known. These include the distorted coiled "tuba" and the protruded radial ribs that can be found in micro-landsnails of the genus *Plectostoma*. Here, we experimentally tested whether these shell traits may act as defensive adaptations against predators. First, we identified the predators, namely, *Atopos* slugs and *Pteroptyx* beetle larvae, and their predatory strategies towards *Plectostoma* snails. Then, we characterised and quantified the possible anti-predation behaviour and shell traits of *Plectostoma* snails both in terms of their properties and efficiencies in defending against the *Atopos* slug predatory strategies, namely, shell-apertural entry and shell-drilling. The results showed that *Atopos* slugs would first attack the snail by shell-apertural entry, and, should this fail, shift to the energetically more costly shell-drilling strategy. We found that the shell tuba of *Plectostoma* snails is an effective defensive trait against shell-apertural entry attack. None of the snail traits, such as resting behaviour, shell thickness, shell tuba shape, shell rib density and intensity can protect the snail from the slug's shell-drilling attack. However, these traits could increase the predation costs to the slug. Further analysis on the shell traits revealed that the lack of effectiveness these anti-predation shell traits may be caused by a functional trade-off between shell traits under selection of two different predatory strategies. Lastly, we discuss our results in the framework of Red Queen predator-prey coevolution and escalation, and propose several key elements for future study.

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16 **Introduction**
17 Predator-prey interactions are among the key ecological interactions that shape the diversity of
18 biological form (Vermeij, 1987). Predation may drive the evolution of prey morphology, as prey

19 forms that possess anti-predator characteristics increase survival and are selected under predation
20 selection pressure. This selection acts either unidirectionally – escalation that only drives the
21 evolution of the prey; or reciprocally – Red Queen coevolution that drives the evolution of both
22 prey and predator (Vermeij, 1994). Such patterns of predator-prey coevolution and escalation
23 have become favourite subjects in the evolutionary biology of biological form.

24 Among the studied prey traits, those of snail shells, which act like armours, have been popular
25 examples in demonstrating anti-predation adaptation (Vermeij, 1993). Among the reasons for this
26 popularity are the fact that the shell is a conspicuous external structure, and the fact that its anti-
27 predation properties may be observed directly as compared to other non-morphological anti-
28 predation traits. Also, the interaction between predator and snail and the effectiveness of the anti-
29 predation traits of the shell can be studied indirectly by examining traces and marks of both
30 successful and unsuccessful predation on the shells (Vermeij, 1982; Vermeij, 1993). More
31 importantly, the predator-prey interaction and evolution can be traced over time because shells
32 with those predation marks are preserved in the fossils record (Alexander & Dietl, 2003; Kelley
33 & Hansen, 2003).

34 The adaptive significance of shell anti-predation traits is better known for marine snails than for
35 land snails (Goodfriend, 1986; Vermeij, 1993). This does not mean that land snails are less likely
36 to be preyed upon in terrestrial ecosystems as compared to the marine ecosystems. In fact, the
37 terrestrial ecosystem is a hostile environment to land snails, who face a taxonomically wide range
38 of predators (Barker, 2004 and reference therein). The fact that molluscs have diversified to
39 become the second largest phylum on land after the arthropods, suggests that land snails have
40 evolved successful adaptations to deal with predation, and the evolution of shell morphology is
41 likely to have played an important part.

42 The land snail shell is a single piece of coiled exoskeleton that consists of several layers of
43 calcium carbonate. Its basic ontogeny follows a straightforward accretionary growth. Shell
44 material is secreted by the mantle, which is located around the shell aperture, and is added to the
45 existing aperture margin. Despite this general shell ontogeny that produces the basic coiled shell
46 of all land snails, there is a great diversity of shell forms.

47 Many of the shell traits of land snails (e.g., whorl number and size, shell periphery form,
48 umbilicus, shell coiling direction, aperture shape and size, and shell shape, thickness and size) are
49 adaptive responses to abiotic ecological factors; by contrast, very few traits, viz. aperture shape
50 and size, shell size, and shell wall thickness, are known to offer a selective advantage when faced
51 with predation (Goodfriend, 1986). Since Goodfriend's (1986) review, few additional studies
52 have shown the adaptive significance of land snail shell traits under predation pressure, namely,
53 aperture form by Gittenberger (1996), Quensen and Woodruff (1997), Hoso (2012) and Wada and
54 Chiba (2013); shell form by Quensen and Woodruff (1997), Schilthuizen et al. (2006), Moreno-
55 Rueda (2009) and Olson and Hearty (2010); shell ribs by Quensen and Woodruff (1997); and
56 shell coiling direction by Hoso and Hori (2008).

57 Conspicuously lacking from this list are protruding radial ribs and distorted-coiling of the last
58 whorl. These traits have been shown to have anti-predation function in marine snails (Vermeij,
59 1993; Allmon, 2011), but it remains unclear whether the same is true for land snails, where such
60 traits are less common (Vermeij & Covich, 1978). Probably the only land snail taxon that
61 possesses both of these traits is the genus *Plectostoma* (Figure 2E). Some *Plectostoma* species
62 have a regularly-coiled, dextral shell throughout their ontogeny, similar to most of the other

63 gastropods. However, many *Plectostoma* species are unusual in having a shell that coils dextrally
64 at the beginning of shell ontogeny (hereafter termed 'spire'), then changes direction at the
65 transitional shell part (hereafter termed 'constriction'), and finally forms a last whorl that is
66 detached from the spire and coils in an opposite direction (hereafter termed 'tuba'; van Benthem
67 Jutting, 1952; Vermeulen, 1994). Similar morphological transitions during shell ontogeny are
68 known for other extant and fossil molluscs (e.g. Okamoto, 1988; Clements et al., 2008). In
69 addition to this irregular coiling, there is great diversity in the shell radial ribs of *Plectostoma* in
70 terms of density, shape, and intensity (van Benthem Jutting, 1952; Vermeulen, 1994). Clearly,
71 *Plectostoma* is a good model taxon to improve our understanding of the ecological function of
72 both of these unusual shell traits.

73 This study was designed to test the anti-predation functions of *Plectostoma* shell traits.
74 Specifically, we investigated the association of *Plectostoma* shell traits with its predator's
75 predatory behaviour to improve our understanding of the anti-predation significance of the shell
76 ribs and distorted coiling. In order to do this, we first revealed the predatory behaviours of
77 *Atopos* slugs and Lampyridae beetle larvae, which are the main predators for *Plectostoma*, based
78 on the data obtained from literature and our own experiments. Next, we tested several hypotheses
79 regarding the adaptive significance of these shell traits against the predatory behaviour of *Atopos*,
80 but not Lampyridae larvae, because we could not obtain sufficient material of the latter predator
81 for experiments. Additionally, we discuss the results of this study in the context of predator-prey
82 interaction and shell trait evolution.

83 ## Materials and Methods
84 ### Ethics Statement
85 The permissions for the work in the study sites were given by the Wildlife Department of Sabah
86 (JHL.600-6/1 JLD.6, JHL.6000.6/1/2 JLD.8) and the Economic Planning Unit, Malaysia (UPE:
87 40/200/19/2524).

88 ### Field observation and laboratory observation
89 We compiled all the data regarding the interaction between *Plectostoma* and its predators from
90 our field observations conducted between October 2002 and January 2013 in Peninsular Malaysia
91 and Sabah. Most of these observations were made during the day time. Whenever possible, field
92 notes and photographs were taken when interactions between *Plectostoma* species and their
93 predators were seen.

94 ### Literature review
95 In addition to the field observations, we compiled published literature on the predatory behaviour
96 towards land snails for the two predators that were identified from our field observations, namely
97 Rathouisiidae slugs and Lampyridae beetle larvae. We used the search engines of Web of Science
98 and Google Scholar on 23rd May 2013, with the keywords (rathouis* AND snail*) and (lampyrid*
99 AND snail*).

100 ### Predation tests
101 On the basis of the field observations and literature review described above, we identified two
102 predatory strategies, namely, shell-apertural entry and shell-drilling (Figure 1). Under the
103 assumption that predators drive the evolution of prey traits, we hypothesized one behavioural and
104 three shell traits that may protect *Plectostoma* against both predatory strategies, namely: resting
105 position, radial rib density and intensity, tuba length and circumference, and shell thickness. If

106 these traits are adaptations resulting from evolutionary arms races or escalation, we would expect
107 an interaction with the predators' behaviour. So, we conducted two sets of tests, each of them
108 consisting of several subtests to evaluate the anti-predation hypothesis of the *Plectostoma* shell
109 traits under the respective predatory strategy of *Atopos* slugs (Figure 1). Our analysis focused on
110 *Atopos* and several populations and species of *Plectostoma* from two nearby limestone hills in
111 Sabah, Malaysia. No further tests were done on Lampyridae beetle larvae because we did not
112 obtain sufficient beetle larvae.

113 **Test 1: *Plectostoma* snails' anti-predation traits against *Atopos* slug shell-drilling behaviour.**
114 To date, only one direct field observation of shell-drilling by *Atopos* is available (Table 1). To
115 obtain more data on this predatory behaviour, we carried out several tests. First, we investigated
116 drill holes on the *Plectostoma* shell made by *Atopos* to evaluate whether the drill hole is
117 distinctive and conveys biological information, such as proboscis size [Test 1 (a)].

118 Once the reliability of the drill hole in characterising the slug's drilling behaviour was confirmed,
119 we tested the effectiveness of several hypothetical *Plectostoma* shell traits which could have anti-
120 predation function, namely, shell tuba [Test 1 (b)], ribdensity and intensity [Test 1 (c)], and shell
121 thickness [Test 1 (d)].

122 **Test 1 (a) – *Atopos* drill hole characteristics on the shell of adult *Plectostoma*.**
123 An *Atopos* slug with a body length of 14 mm, was collected from the rock face of Batu Kampung
124 (5° 32'11"N, 118°12'47"E, hereafter Site A) (Figure 1C, No. 5 in Table 1). At the same time, 250
125 living adult and juvenile *P. concinnum* were collected from the same location. After that, the
126 *Atopos* and the *P. concinnum* snails were kept in a tank (30 cm × 30 cm × 14 cm). The micro-
127 habitat in the tank was set up to mimic the natural habitat at site A, and consisted of limestone
128 rock pieces and temperature (25°C - 30°C) and humidity (95% - 100%) control. During the test,
129 which lasted from 19th December 2011 to 24th February 2012, we regularly collected empty shells
130 of dead *Plectostoma* from the tank. Adult empty shells with drill holes were retained for analysis.
131 Empty shells without drill holes were discarded as the cause of death cannot be ascertained. The
132 test ended when the *Atopos* was no longer seen, and presumably dead. The diameter and position
133 of drill holes on the shells were examined and the number of ribs of each shell was quantified.

134 **Test 1 (b) – Association between slug shell-drilling, and adult snail shell tuba and rib density.**
135 Like in marine predator-snail interactions, where predators tend to drill a hole at less-ornamented
136 positions of the prey shell (Kelley & Hansen, 2003) we may expect *Atopos* to drill its holes
137 preferentially between shell ribs, rather than through them. Conversely, if snail shell ribs are
138 adaptive traits in the context of the slug's shell-drilling behaviour, we would expect the snail shell
139 to have evolved more densely-placed, thicker, and more protruded ribs.

140 To examine the association between shell rib density and drill hole position, we studied
141 *Plectostoma* shell specimens from museum collections collected from two limestone outcrops
142 (Batu Kampung – near Site A, and Batu Tomanggong Besar (5°32'3"N 118°23'1"E)). These two
143 limestone outcrops support dense *Plectostoma* populations, which show high variability in shell
144 rib density. We selected museum specimens that belongs to two samples (i.e. populations) from
145 Batu Kampung (*P. concinnum*, collection numbers BOR 1690, BOR 2196), and 9 samples (i.e.
146 populations) from Batu Tomanggong Besar (collection numbers RMNH.MOL 330506; *P*. cf.
147 *inornatum*: Samples T29, T33, T34, and T45; *P. fraternum*: Samples T7, T21, T22, and T42; and
148 *P*. cf. *fraternum*: Sample T 44). All were collected between April 2002 and January 2004.

149 Each of the samples consists of *Plectostoma* empty shells collected beneath the rock face where
150 living *Plectostoma* individuals were also found. For each sample, shells with a characteristic
151 *Atopos* drill hole were selected for analysis. We divided the shells into two groups based on the
152 drill hole position: 1) hole directly through the shell wall and located between two ribs (hereafter
153 BETWEEN RIBS), and 2) hole drilled through one or two ribs as well as the shell wall (hereafter
154 ON RIBS). The two groups were used as the dependent variable, and were binary scored as (1)
155 for BETWEEN RIBS and (0) for ON RIBS. In addition, we identified three predictor variables
156 that may influence the slug drilling behaviour. First, the slug proboscis size, which was measured
157 as the greatest diameter (mm) for circular and slightly oval drill holes (hereafter HOLE SIZE).
158 Second, the rib density of the shell which was quantified as the total number of ribs on the shell
159 (hereafter RIB DENSITY) because all shells a similar number of whorls (mean: 5.15, SD: 0.35;
160 Supplementary materials File S1, Page 22: Table S2). Lastly, the random chance – the probability
161 that a hole was made in between ribs, which is related to the HOLE SIZE and RIB DENSITY.
162 For example, by random chance, a slug with a narrow proboscis (i.e., low HOLE SIZE) has a
163 greater probability to drill a hole in between the ribs on a shell that has fewer ribs (low RIBS
164 DENSITY) because more rib spacings that are larger than the slug proboscis size are available.
165 Thus, we counted total number of rib spacings larger than HOLE SIZE (hereafter CHANCES).

166 We used a logistic regression to model the likelihood that the slug drills a hole either BETWEEN
167 RIBS or ON RIBS as a function of HOLE SIZE, RIB DENSITY, and CHANCES (i.e., Predicted
168 logit of (BETWEEN RIBS) = $\beta_0 + \beta_1$*(HOLE SIZE) + β_2*(RIB DENSITY) + β_3*(CHANCES).
169 Our objective was to investigate the amounts of variance attributable to each predictor variable.
170 The analysis was done in R statistical package 2.15.1 (R Core Team, 2012) and the R scripts can
171 be found in Supplementary materials File S2.

172 **Test 1 (c) – Correlation between *Plectostoma* shell rib density and rib intensity.**
173 In addition to rib density, it is essential to quantify the amount of shell material that *Plectostoma*
174 snails invest to grow thick and protruded ribs (hereafter rib intensity). However, we cannot
175 quantify this from the same shell remains that we had used in test 1(b) because the shell ribs of
176 these specimens were heavy eroded. Thus, we analysed rib intensity from 14 preserved
177 *Plectostoma* individuals that were collected alive from the same rock face at Batu Kampung and
178 Tomanggong Besar, where the shell remains were collected (collection number RMNH 330508;
179 T 21 (n = 3), T 22 (n = 1), T 42 (n = 2), T 7 (n = 1), T 44 (n = 1), BOR 2991 (n = 3), T 33 (n =
180 3)). These 14 shells have different ribs density (47 – 138 ribs per shell), which spans the broadest
181 possible range of rib density, and have the most intact ribs on the shell.

182 We used X-ray microtomography (µCT) to estimate the amount of shell material that
183 *Plectostoma* invests in rib growth (Figure 3). First, we obtained a series of X-ray tomographies of
184 each shell with a high-resolution SkyScan 1172 (Aartselaar, Belgium). The scan conditions were:
185 60 kV; pixels: 668 rows X 1000 columns; camera binning 4 X 4; image pixel size 7 – 9 µm;
186 rotation step 0.5°; rotation 360° (Step 1 in Figure 3).

187 Then, we reconstructed 2D grey scale images (i.e. cross-sections) from X-ray tomography series
188 with NRecon 1.66 (©SkyScan). The settings were: beam-hardening correction 100% and ring
189 artifacts reduction 20. Next, these 2D images were transformed to the final half-tone binary
190 images for each shell in CTAnalyser 1.12 (©SkyScan). This was done by filtering out grayscale
191 index <70. At this stage, each shell was represented by hundreds of 2D cross-section binary
192 images (Step 2 in Figure 3).

193 Each of these 2D images consisted of white and black pixels, where the white pixels represent the
194 solid shell material (shell together with ribs) and the black pixels are background or lumen. When
195 the series of cross-section images was analysed, the total voxels which represent the shell
196 material volume could be determined. Hence, we analysed the volume of shell material from two
197 datasets of each shell. The first was the original 2D cross-section binary images which represent
198 the total volume of shell material contained in whorls and ribs (Step 3 in Figure 3). The second
199 was the volume of shell materialcontained in the shell whorls only, after removal of the shell ribs
200 from each cross-section image. The latter was done manually by changing white rib-pixels into
201 black ones in Paint (©Microsoft Windows 7) (Step 4 in Figure 3). After that, the volume of shell
202 material was calculated for both datasets with Individual 3D object analysis, as implemented in
203 CTAnalyser 1.12 (©SkyScan) (Step 5 in Figure 3). Finally, the rib intensity (i.e. amount of shell
204 material in the ribs) was calculated by subtracting the volume after rib removal from the the total
205 volume with ribs included (Step 6 Figure 3).

206 We wished to test if there is a significant correlation between rib intensity and number of ribs.
207 However, as there is variability in the shell size for the shells that vary in rib density, we
208 quantified a set of size variables of the shell (number of whorls, height, width, and volume of
209 shell material of the shell whorls after rib removal, and then checked for confounding effects of
210 shell size variables with the anti-predation shell traits. The results showed that only one of the
211 shell size variables, i.e. the volume of shell material after rib removal, is significantly correlated
212 with the anti-predation shell traits (Supplementary materials File S1, Page 23: Table S3).

213 So, we also ran an additional partial correlation test between the same two variables (rib intensity
214 vs. number of ribs) after controlling for total volume of shell material after rib removal, to
215 account for confounding effects of the shell size difference. Pearson correlations were performed
216 in the two tests as all variables were normally distributed (Shapiro-Wilk normality test, $p > 0.05$)
217 with R statistical package 2.15.1 (R Core Team, 2012) and R scripts can be found in
218 Supplementary materials File S2.

219 **Test 1 (d) – Relationships between shell thickness, rib number, and shell size.**
220 We obtained 3D models (PLY format) of each of the 14 shells by using the original 2D cross-
221 section binary images that were obtained from experiment 1(c). After that, we measured the shell
222 thickness of the last spire whorl by making a cross-section of the digital 3D models with Blender
223 2.63 (Blender Foundation, www.blender.org). We obtained the shell thickness data from the
224 digital 3D models instead of the actual specimens because it is difficult to make a clean cross-
225 section on this tiny shell.

226 In order to assess if the prey invests more shell material in increasing the shell thickness, when it
227 invests less in the ribs, we tested the correlation between shell thickness and number of ribs.
228 Similar to test 1(c), we also ran an additional partial correlation test between the same two
229 variables after controlling for the volume of shell material after rib removal, to account for the
230 variability in shell size differences. In addition, the relationships between shell thickness, rib
231 number, and shell size were explored. Pearson correlations were performed in these tests as all
232 variables were normally distributed (Shapiro-Wilk normality test, $p > 0.05$) in R statistical
233 package 2.15.1 (R Core Team, 2012) and R scripts can be found in Supplementary materials File
234 S2.

235 **Test 2: *Plectostoma* snails' anti-predation traits against the apertural-entry behaviour of the *Atopos* slug**

236   Based on the literature review and our tests 1(a) and 1(b), we know that *Atopos* would use its
237   shell-apertural entry strategy whenever possible, but would shift to shell-drilling if the initial
238   entry strategy failed. To enter via the aperture, the slug inserts its feeding apparatus and passes it
239   through the shell body whorl, apparently pushing aside the operculum, to reach the soft body of
240   the prey. Thus, traits of the predator's feeding apparatus, such as its length, size, and flexibility,
241   are the key to accomplishing predation.

242   Under a hypothetical anti-predation adaptation scenario, *Plectostoma* would have evolved to
243   defend itself behaviourally and/or shell-morphologically against shell-apertural entry by *Atopos*.
244   Thus, we tested the anti-predation effectiveness of the possible behavioural and morphological
245   adaptations of *Plectostoma*.

246   **Test 2 (a) – Predator preferences for three different prey shell forms.**
247   Two *Atopos* slugs, with body lengths of 7 and 15 mm, were collected from Site A (No. 7 & 8 in
248   Table 1). Each of the slugs was kept in a plastic box (12 cm X 8 cm X 7.5 cm), which contained a
249   piece of limestone rock and its temperature and humidity were controlled as in experiment 1 (a).
250   The boxes were kept under the table in a room with opened window to simulate the natural
251   habitat for the slugs that are active nocturnally and rest in a shaded place during the daytime.

252   Live *P. concinnum* individuals were collected from the site A for this test. For each experiment,
253   three individuals were placed on the rock in the plastic boxes. These three preys represented three
254   different shell forms (i.e. growth stages): 1) shell with no tuba and peristome lip (juvenile, e.g.
255   Figure 4A: shells e – g), 2) shell with partial tuba but no peristome lip (sub-adult, e.g. Figure 4A:
256   shells h – j), and 3) shell with fully grown tuba and peristome lip (adult, e.g. Figure 4A: shell l).
257   During the experiment, the interactions between predator and prey were checked every 3 hours to
258   minimise the disturbance to the organisms. Each experiment ended after the slug was observed
259   inactive (i.e. hiding under the rock) and at least one of the prey was consumed. After that, the
260   three prey shells were removed for further analysis, and replaced with another three living snails
261   to start a new experiment.

262   We ran nine such experiments, one with slug No.7 and eight with slug No. 8. After each
263   experiment, each of the three shell forms was scored as having either survived or died. Also, the
264   shell of each dead prey was examined for possible traces left by slug predation. In addition, we
265   also estimated the predator's attack and consuming time from the time intervals between the
266   moments when all prey were last seen alive and the moment the experiment was ended. The total
267   of prey that died from slug attack in each of the three shell form categories was summed up from
268   all experiments. Lastly, we tested if all three shell forms were equally likely to be killed by the
269   predator by using chi-squared test (goodness of fit) in R statistical package 2.15.1 (R Core Team,
270   2012) and R scripts can be found in Supplementary materials File S2.

271   **Test 2 (b) – Effectiveness of resting behaviour of *Plectostoma* snails against *Atopos* shell-apertural entry**
272   **predatory behaviour.**
273   When a *Plectostoma* snail is resting or is disturbed, it withdraws its soft body into the shell and
274   adheres its shell aperture to the substrate. Thus, when the snail is in this position, its aperture is
275   not accessible to the slug, and for the slug to access the shell aperture, it would need to remove
276   the shell from the substrate. In this test, the ability of the slug to manipulate the adherent prey
277   shell was inferred by examining the drill hole location of the specimens used in Test 1(b). We
278   predict that the sector of the shell facing the substrate is less susceptible to drilling by the slug if
279   it is unable remove the adherent prey shell from the substrate.

280 For each of 133 shells, we recorded the location of the drill hole. We divided drill-hole locations
281 of these shells into four categories, which represent different sectors, namely: A) shell whorls that
282 face the substrate; B) shell whorls that face the tuba; C) shell whorls opposite (A); and D) shell
283 whorls opposite (B) (Figure 9A). Then, we tested if all four sectors of shell whorls are equally
284 susceptible to slug drilling by using chi-squared test (goodness-of-fit). We also tested if the rib
285 density (indicating prey defence), and drill hole size (indicating predator size), differ among these
286 four categories with Kruskal-Wallis rank sum test (kruskal.test). All statistical analyses were done
287 in R 2.15.1 (R Core Team, 2012) and R scripts can be found in Supplementary materials File S2.

288 **Test 2 (c) – Effectiveness of prey's shell whorl morphometrics against shell-apertural entry by *Atopos***
289 **proboscis.**
290 When a *Plectostoma* snail withdraws into its shell, part of the lower shell whorls are left vacant.
291 We named this vacant part the 'predatory path', located between shell aperture and soft-body
292 withdrawal terminal point (i.e. between the endpoint of the shell whorls and the withdrawn snail's
293 operculum). In shell-apertural entry predation events, the predator's feeding apparatus would
294 need to pass through the predatory path to reach the snail that is withdrawn deeply into the shell.
295 Hence, success of a predation event would depend on the interplay between the morphometrics of
296 both the prey's predatory path and the predator's feeding apparatus. In this section, we quantified
297 these morphometrics. Because both prey and predator traits vary throughout their growth, we
298 assessed variability of these morphometrics at several different growth stages.

299 For the predatory path analysis, we selected from site A, 11 living snails representing a range of
300 shell developmental stages (Figure 4A). Then, in the field, we disturbed each snail with a forceps
301 so that the animal withdrew into the shell. Immediately after that, the snail was killed with and
302 preserved in 70% ethanol. After arriving in the laboratory, we photographed each specimen to
303 record the withdrawal position of the animal in its translucent shell. Then, we obtained 3D
304 models (PLY format) of these shells, based on the X-ray microtomography (μCT) technique as
305 described in Test 1(c), using CTAnalyser 1.12 (©SkyScan).

306 After the 3D models were obtained, we extracted the whole predatory path from the 3D model of
307 an adult shell (hereafter "reference shell"). This is the shortest possible path when traveling inside
308 the shell whorls from the aperture in the direction of the apex of the adult shell (Figure 4B). We
309 also extracted from the reference shell the whole shell ontogeny axis, which represents the entire
310 shell's growth (Figure 4C). Next, we determined the terminal withdrawal point for each
311 corresponding growth stage from the photographs and 3D models of the 11 shells (Figure 4D).
312 After that, we calculated the distance of the portion of the whole predatory path which
313 corresponded to the predatory path for each the 11 growth stages, and plotted these predatory
314 path distances on the ontogeny axis (Figure 4E). Then, we described the geometry of the shell
315 whorls as a 3D spiral, in the terms of torsion and radius of curvature (Harary & Tal, 2011), which
316 were used to explore the geometry of the whorls along the predatory path.

317 Then, we performed the morphometrics of the slug's proboscis. However, we could not obtain an
318 accurate measurement for the length of a fully extended proboscis because we were limited by
319 the small number of *Atopos* specimens and the fact that the proboscis was not fully extended in
320 most preserved specimens. Nevertheless, we attempted to estimate the length of the proboscis
321 based on the following facts and assumptions: (1) we know that the drill hole size corresponds to
322 *Atopos* body size and proboscis diameter (Test 1(a), Kurozumi, 1985; Wu et al. 2006); (2) we
323 know the maximum and minimum sizes of the drill holes from Test 1(b) are 0.13 mm and 0.33

324   mm, which represent the range of proboscis diameters of *Atopos* in Site A and Tomanggong
325   Besar; and (3) we assume that the dimension (i.e. diameter × length) of our slug proboscis is
326   similar to those published for *Atopos kempii* (Ghosh, 1913: Plate X) (Figures 10A and 10B).
327   Based on this information, we estimated that the minimum and maximum dimensions of the
328   proboscis are 0.13 x 0.8 mm and 0.33 x 1.7 mm.

329   Finally, we overlaid the shell predatory path with the slug proboscis morphometrics across the
330   ontogenetic trajectory. We evaluated the growth stages for which the prey shells are not
331   susceptible to the predator's shell-apertural entry, by comparing the morphometrics for the prey
332   predatory path with the predator proboscis. To do this, we considered that preyis safe from the
333   predator when the distance of the predatory path is longer than the predator's proboscis length
334   and when prey's radius of curvature is smaller than predator's proboscis diameter, so that
335   predator's proboscis is too large to enter the shell.

Results
Predators and their behaviour towards *Plectostoma* based on direct observation in the field

338   We made five direct observations on the interactions between *Plectostoma* snails and their
339   predators (Table 1). We found two *Pteroptyx* species larvae (Lampyridae) and an *Atopos* slug
340   species (Rathouisiidae) attacking three *Plectostoma* species. *Pteroptyx* was seen to attack adult
341   and juvenile *Plectostoma* snails by shell-apertural entry (Figures 2A and 2B) whereas *Atopos*
342   were seen to attack adult *Plectostoma* snails by shell-drilling (Figure 2C).

Literature survey of behaviour of Lampyridae beetle larvae and Rathouisiidae slugs towards land snails

345   We could not find any literature regarding to the predatory behaviour of the species *Pteroptyx* cf.
346   *valida* and *Pteroptyx tener* on land snails. Nevertheless, beetle larvae of other genera in
347   Lampyridae were recorded to attack land snails varying in size (the smallest being 2 mm) by
348   shell-apertural entry (Table 2), in which the larva inserts its elongate head into the shell via the
349   shell aperture.

350   Published information on the *Atopos* slug's predatory behaviour towards land snails was similarly
351   scarce (Table 3). Despite this, other genera in the Rathouisiidae are reported to use two different
352   predatory strategies to attack land snails, namely, shell-apertural entry and shell-drilling (Table
353   3).

354   The following is a summary of rathouisiid behaviour as we distilled it from literature (Table 3).
355   When attacking a large prey snail with a large shell aperture, Rathouisiidae slugs move into the
356   shell via aperture and attack the soft body that has withdrawn deep into the shell. In cases where
357   the aperture is too small for the slug to enter, the slug inserts only its proboscis, via shell aperture,
358   into the shell. Thus, Rathouisiidae slugs would manipulate the small prey shell so that the prey
359   shell aperture would be exposed to the slug proboscis.

360   However, Rathouisiidae cannot attack a prey item by shell-apertural entry when the opening is
361   absent (such as is the case with snail eggs) or obstructed. In this situation, the slugs would drill a
362   hole into the prey shell and then the slug would insert its proboscis, via the drill hole. The drill
363   hole is either circular or oval in shape, and the size of the drill hole is related to the size of the
364   slugand has a distinctive narrow scraped rim around the margin.

365 Rathouisiidae consume the prey snail by digesting the soft body in the prey shell and taking up
366 the dissolved snail with its proboscis. During the consumption, the slugs hold the prey tightly
367 with the foot in a distinctive posture.

First set of tests: (1) *Plectostoma* anti-predatory traits against *Atopos* shell-drilling behaviour.
368
369 **Test 1 (a) – Characteristic drill holes in the shell of *Plectostoma* adult snails.**
370 We found drill holes made by an *Atopos* slug, in six empty *Plectostoma concinnum* shells
371 (Supplementary materials File S1, Page 1: Table S1, Figure S1). The experimental slug did not
372 show any stereotyped choice of drill location on the shells. As shown in Figures 2E and 2F, these
373 drill holes are distinctive with a narrow scraped rim around the margin. All the drill holes that
374 were made by the same slug had uniform size (mean diameter = 0.21 mm, SD = 0.01 mm, n = 6).
375 Of these six prey shells, two had the drill hole located in between two ribs and four had the drill
376 hole through the ribs. The number of ribs of the six shell vary from 93 to 108 (mean = 98, SD =
377 6, n = 6).

378 **Test 1 (b) – Association between slug shell-drilling behaviour and adult snail shell tuba and rib density.**
379 The drill hole diameters of the 133 prey shells varied between 0.13 mm and 0.33 mm (mean =
380 0.230 mm, SD = 0.045, n = 133; Supplementary materials File S1, Page 2 – 19: Figure S2 - S12).
381 Four of these (3%) had two drill holes, one on the tuba and another on the spire (Supplementary
382 materials File S1, page 20 – 21: Figure S13). The drill hole of 70 shells (53%) was made through
383 the ribs (ON RIBS), whereas the drill hole of the other 63 shells (47%) was made in between the
384 ribs (BETWEEN RIBS). The result showed a logistic model that was more effective than the
385 null model as follows: Predicted logit of (BETWEEN RIBS) = 10.448 - 11.316*(HOLE SIZE) -
386 0.095*(RIBS DENSITY) + 0.033*(CHANCES), (AIC = 83.382; χ^2 = 109.63, df = 3, p = 0).
387 According to the model, the statistically significant coefficients were for intercept (β_0 = 10.448, Z
388 = 2.867, p = 0.001) and RIB DENSITY (β_2 = -0.0916, p < 0.0005; Odds Ratio = 0.91, CI = 0.87-
389 0.95). The number of available space for drilling in between ribs (CHANCES) and the slug size
390 (HOLE SIZE) were not significant (p > 0.1). In other words, the slug is less likely to drill a hole
391 through the ribs on a densely ribbed shell, and this tendency is independent from slug size and
392 chance.

393 **Test 1 (c) - Correlation between rib density and rib intensity of *Plectostoma*.**
394 Different *Plectostoma* species and populations exhibit high variability in the rib density, ranging
395 from 49 ribs to 154 ribs per shell. There is a significant negative correlation between the rib
396 intensity and the number of ribs of the shell (Figure 6A; r = - 0.95, t = -10.74, df = 12, p < 0.001;
397 Supplementary materials File S1, Page 22 and 24: Table S2, Figure S14). Both rib intensity and
398 number of ribs are strongly correlated with the amount of shell materials after removal of the ribs
399 (= shell size) (Supplementary materials File S1, Page 25: Figure S15 and S16). Nevertheless,
400 after controlling for this, there is still a significant negative correlation between rib intensity and
401 number of ribs on the shell (Figure 6B; r = - 0.63, t = -2.71, n = 14, p < 0.001). These results
402 indicate that there is a statistically significant trade-off between rib density and rib intensity,
403 irrespective of shell size.

404 **Test 1 (d) – Variation of shell thickness of *Plectostoma* with varying shell size and number of ribs.**
405 Different *Plectostoma* populations and species have different shell thicknesses, ranging between
406 0.29 mm and 0.46 mm. There is a significant negative correlation between shell thickness and
407 number of ribs (Figure 7A; r = - 0.73, t = -3.70, df = 12, p < 0.005; Supplementary materials File
408 S1, Page 22: Table S2). Shell thickness is strongly correlated with the amount of shell materials
409 after removal of the ribs (= shell size) (Supplementary materials File S1, Page 26: Figure S17).
410 After controlling for this, there is no significant correlation between the shell thickness and the

411 number of ribs on the shell (Figure 7B; r = 0.06, t = -0.192, n = 14, p =0.85). Thus, larger
412 *Plectostoma* shells simply are thicker.

413 **Second set of tests: (2) Anti-predation traits in *Plectostoma* against shell-apertural entry**
414 **behaviour of *Atopos*.**
415 **Test 2 (a) – Predator preference for different prey shell growth stages.**
416 Table 4 shows the snails of three ontogenetic categories that did and did not survive. It shows that
417 the slugs prefer to attack and consume prey with an incomplete tuba or no tuba at all (Table 4; χ^2
418 = 8.4, df = 2, p < 0.05; Supplementary materials File S1, Page 27 – 29: Table S4, Figure S18). In
419 all tests, adults with a complete tuba and peristome survived shell-apertural entry.
420 The predatory behaviour of the slug could not be observed directly because the slug proved very
421 sensitive to disturbance and light. Shells of consumed prey did not show any drill-holes, which
422 suggests that the slug attacked the juvenile prey via the shell aperture. Furthermore, 11 out of the
423 15 predated shells still had an intact operculum attached to the posterior side of the shell aperture
424 (Figure 8). It is likely that the slug could took at least seven hours to attack and consume the
425 entire soft body of juvenile and sub-adult prey (Test no. 12 in Table 4).

426 **Test 2 (b) – Effectiveness of resting behaviour of *Plectostoma* snails against *Atopos* shell-apertural entry**
427 **predatory behaviour.**
428 Our data show that the four sectors of the shell differ in their susceptibility to drilling by the slug
429 (Figures 9A and 9B; χ^2 = 22.1, df = 3, p < 0.0001; Supplementary materials File S1, Page 30:
430 Figure S19). Drill hole frequency is highest in sectors A and B (both 35%), and lowest in sectors
431 C and D (18% and 12%, respectively). The high frequency of drill holes in sector A suggests that
432 the slug is capable of removing adult prey from the substrate. The drill hole size (representing
433 predator size) is not significantly different among the sectors(Figure 9C; Kruskal-Wallis χ^2 =
434 3.71, df = 3, p = 0.29). This indicates that slugs of all ages and sizes are capable of manipulating
435 the prey. Furthermore, prey shell rib densities are not significantly different among the four
436 categories (Figure 9D; Kruskal-Wallis χ^2 = 7.17, df = 3, p = 0.06), which suggests that the slug's
437 ability to manipulate the prey is not influenced by the prey rib density.

438 **Test 2 (c) – Effectiveness of shell morphometrics against shell-apertural entry by the *Atopos* proboscis.**
439 Radius of curvature (a proxy for whorl diameter) of the prey shell increases constantly with slight
440 fluctuations throughout the shell ontogeny, apart from a few short but dramatic changes at the
441 constriction (Figures 10A and 10B, 11; Supplementary materials File S1, Page 31: Figure S20).
442 In addition, the predatory distance of the prey shell increases exponentially as the shell grows
443 (Figures 10A, 10B, Supplementary materials File S1, Page 31: Figure S21). In addition to these
444 two morphometric changes throughout shell ontogeny, there is a dramatic change in torsion
445 between the spire whorls and the tuba whorl (Figure 11, Supplementary materials File S1, Page
446 32: Figure S22).

447 When the hypothetical slug proboscis morphometrics are plotted together with prey shell
448 morphometrics, it becomes clear that a snail that has grown to at least five whorls would be safe
449 from shell-apertural entry attacks by the smallest *Atopos* slug (green box in Figure 10A).
450 Although the slug's proboscis could fit into the whorls (proboscis diameter < radius of curvature
451 of prey shell, Figure 10A), it is too short to reach the soft body of an animal that has at least 5
452 spire whorls (slug proboscis length < predatory path distance of prey shell, Figure 10A).
453 However, a larger slug could attack and consume larger prey by shell-apertural entry. A larger
454 slug could attack prey with more than 5 spire whorls and also prey with a partial tuba because of
455 the increase in its proboscis length and diameter (Figure 10B). Eventually, only fully-grown prey

456 with a complete tuba would remain safe from shell-apertural attack of a fully-grown *Atopos* slug
457 (green box in Figure 10B).

Discussion
Predatory behaviour of *Atopos* slugs toward *Plectostoma* micro-landsnails.
460 In general, our results show that in attacking and consuming the unusually-shaped *Plectostoma*,
461 the slug *Atopos* uses the same predatory strategies that are widespread in other members of the
462 slug family Rathouisiidae. The *Atopos* population in this study was found on humid and shaded
463 limestone rock surfaces. In suitable habitat, up to 15 slugs could be found in 25 m^2 of rock face
464 (no. 1 in Table 1). The slug is a nocturnal predator and it was seen foraging at night and, in shady
465 places, also early in the morning. During the day, the slug probably hides in the cracks of the
466 limestone rock. Similar ecological characteristics have been reported for other Rathouisiidae.

467 *Atopos* proved to be one of the main predators for *Plectostoma* in the two limestone hills in our
468 small study area. Possibly, this is the case in general, because many shells of other *Plectostoma*
469 species throughout the distribution area of the genus have the characteristic drill holes as our
470 studied shells (Borneo, Kinabatangan region: Schilthuizen et al., 2006, and Peninsular Malaysia:
471 Liew Thor-Seng, unpublished data, Supplementary materials File S1, Page 33 – 34: Figure S23).
472 We are not sure whether the slugs in our case are generalist predators that also feed on other snail
473 species, as is the case with other Rathouisiidae slugs (e.g., Table 3), because we have only
474 recorded *Plectostoma* species as prey for *Atopos* in the field so far.

475 Predators need effective strategies to find, pursue, catch, and consume their prey (e.g., Vermeij,
476 1993; Alcock, 1998). Unfortunately, we were unable to study the behaviour leading up to prey
477 attack, because we could obtain only a few live slugs, which are also very sensitive to
478 experimental manipulation. At our two study sites, *Plectostoma* snails have high population
479 density (i.e., Site A, 150 individuals per m^2, Liew Thor-Seng, personal observation, 18th January
480 2013; and Western slope of Batu Tomanggong Besar, 129 individuals per m^2, Schilthuizen et al.,
481 2003). The abundance of *Plectostoma* snails in the vicinity of the places where *Atopos* slugs were
482 found indicates that the slugs can easily find prey. In addition, we also suspect that the slug can
483 effectively pursue their prey, because we observed that *Atopos* crawls faster than *Plectostoma*.

484 During the third stage of predation (prey capture), the prey would withdraw into the shell and
485 adhere its shell aperture to the substrate (e.g. rock surface). The slug would attack by shell-
486 apertural entry by removing the snail from its initial adherent position (Tests 2a & 2b), though we
487 do not know exactly how the slug carries this out. Then, the slug holds the prey tightly in a
488 distinctive posture (Figure 2C, Table 1 and 3). It adheres to the substrate with about two-thirds of
489 the posterior part of the foot, and holds the prey shell with the remaining one-third, which
490 straddles over and lays on the prey shell and pushes the shell against the substrate. On one end,
491 the slug's head lies on the shell aperture or another part of the shell. The other end of the anterior
492 part of the foot, which is slightly lifted from the substrate, has becoming thicker and might act as
493 a pivot point. Thus, it seems to us unlikely that the snail could escape from the strong grip of
494 *Atopos* after having been captured.

495 After the snail has been captured, the slug would attempt to reach the soft body by inserting its
496 proboscis into the prey shell via the shell aperture (Table 3). The slug is more likely to succeed by
497 shell-apertural entry when the prey is not yet fully-grown (Test 2c). All other things being equal,
498 when using the shell-apertural entry strategy, the slug would prefer to attack immature prey over

499 prey with a fully-grown shell (Test 2a). If the slug can reach the deeply-withdrawn body of the
500 snail (lying immediately behind the operculum) it would be able to consume it entirely (Tests 1a
501 & 2a). The slug may take more than three hours to attack and consume a juvenile snail by shell-
502 apertural entry (Test 2a).

503 At the end of consumption, there is hardly any snail tissue left in the prey shell. However, the
504 operculum that had withdrawn together with the soft body into the shell remains intact and has
505 been moved to the outside of the shell (Test 2a). We did not observe how the slug extracts the soft
506 body from the shell, but we suppose the slug may secrete digestive fluid to dissolve the snail's
507 tissues and then ingesting this with its proboscis, like other Rathouisiidae (Table 3). Interestingly,
508 though, these digestive fluids then do not damage the operculum (made from corneous protein)
509 (Test 2a). The operculum is free from physical damage as well (Experiment 2a).

510 The shell-apertural entry strategy would, however, fail if the slug's proboscis cannot reach the
511 withdrawn soft body of snail (Test 2c). In this situation, the slug uses shell-drilling to make a new
512 opening directly on the part of the shell whorls where the snail is hiding (Test 1a). We do not
513 know how much time it takes for the slug to drill a hole on the prey shell. Our results show that
514 the holes made by the same slug individual have the same size (Test 1a) which supports previous
515 studies that found that hole size is related to the slug's proboscis size and therefore to slug size
516 (Table 3). The exact drilling mechanism of the slug remains unknown, but it could be either
517 mechanical or chemo-mechanical because of the narrow scraped rim on the hole margin (Figures
518 2E and 2F).

519 Although Schilthuizen et al. (2006) report that the distribution of holes across the prey shell is
520 characteristic for each slug population, Test 1a shows that this is not due to stereotypical drilling
521 behaviour of the individual slug, since our experimental animal left drill holes on all parts of its
522 prey shells. The slug is able to drill holes either directly on the shell whorl surface or through the
523 ribs (Tests 1a & 1b). Nevertheless, the slug prefers to drill its hole directly on the shell surface,
524 especially in less densely-ribbed shell, and this tendency may not simply be due to a reduced
525 chance of hitting a rib in a shell with larger rib spacing (Test 1b, Figure 5). Indeed, the tendency
526 of the slug to avoid drilling holes through ribs on a less densely ribbed shell suggests that this is
527 because ribs on a less densely ribbed shell are more "intense" (i.e., heavier; Test 1c, Figure 6).
528 This agrees with observations in other drilling snail predators, which also choose the thinnest part
529 of the prey shell for attack (Allmon, Nieh & Norris, 1990; Kelley & Hansen, 2003).

530 In summary, *Atopos* slug might not encounter resistance from *Plectostoma* snail during the first
531 stages of predation. In the final stage, the slug would first attempt its shell-apertural entry strategy
532 to insert its proboscis, and then use the alternative shell-drilling strategy if the first strategy failed.
533 Thus, we conclude that it is likely that *Atopos* slug predation of *Plectostoma* snails is highly
534 successful, even though the slug needs to spend more resources (e.g. time and energy) to
535 neutralise the anti-predation shell traits of the prey. We note that *Atopos* predatory behaviour
536 toward *Plectostoma* micro-landsnails agrees with predatory behaviours of Rathouisiidae slugs to
537 other snails. Hence, predatory behaviour appears to be conserved within the Rathouisiidae.

538 **The effectiveness of anti-predation traits of *Plectostoma* against shell-apertural entry by**
539 ***Atopos*.**
540 The first line of defence of the *Plectostoma* snail against the *Atopos* slug predation is the snail
541 resting behaviour. When snail is resting or disturbed, it withdraws its soft body into the shell and
542 adheres its shell aperture firmly to the substrate. We found that the attachment of the *Plectostoma*

543 shell aperture to the substrate may not be strong enough to resist manipulation by *Atopos*. The
544 slug could remove the snail from the resting position and then approach the shell aperture. Hence,
545 the resting behaviour of the snail is not an effective anti-predation trait against shell-apertural
546 entry.

547 The tuba of a fully-grown shell, however, can act as a second line of defence, as it counteracts
548 shell-apertural entry by creating a longer predatory path than the slug proboscis can traverse.
549 However, our morphometric simulation (Figures 10A and 10B) suggests that survival chances of
550 juvenile snails with incomplete tuba or no tuba at all are slim under shell-apertural attack. Indeed,
551 we have not found any drill holes on the spire of juvenile shells (Test 2a). Our estimation of the
552 *Atopos* proboscis dimensions (i.e. length 0.8 mm - 1.7 mm) agrees with those in other, similar-
553 sized rathouissiids (Kurozumi, 1985: 20 mm long slug with an approximately 2-mm-long
554 proboscis). We would like to point out that our analysis is readily re-evaluated when more data on
555 the anatomy of *Atopos* become available, by simply changing the threshold lines of the proboscis
556 morphometrics in Figures 10 A and 10 B (Supplementary materials File S3).

557 It is worth noting that Lampyridae beetle larvae also use shell-apertural entry to attack
558 *Plectostoma* snails. Hence, the anti-predation properties of the snail tuba against *Atopos* attack
559 might similarly defend against the lampyrid larvae. In addition to the increased predatory path as
560 anti-predation property, it is possible that the twisted vacant tuba whorls also help obstruct the
561 insertion of the feeding apparatus of the slug and beetle larva if these are not flexible enough to
562 pass through the twists of the tuba. In short, this second line of defence posed by the snail tuba
563 could force predators to use an alternative, more costly, predatory strategy.

564 Open-coiled and drastic torsion of the last shell whorl like the tuba in *Plectostoma* snails has
565 evolved several times independently in recent and extinct land and marine snails (Vermeij, 1977;
566 Gittenberger, 1996; Savazzi, 1996). Such shells have a longer predatory path as compared to
567 tightly and regularly logarithmically-coiled shells. We showed that this could be an anti-predation
568 adaptation to shell-apertural entry by the predator (see also Wada & Chiba, 2013), which is
569 opposed to the proposed association between open-coiled shell and low predation pressure (e.g.
570 Vermeij, 1977; Seuss et al. 2012).
571 –

**The effectiveness of *Plectostoma* anti-predation traits against *Atopos* shell-drilling predatory
573 behaviour.**
574 Upon failure of its first attempt at predation by shell-apertural entry, an *Atopos* slug will use the
575 alternative shell-drilling strategy to consume the snail. The slug probably needs to expend more
576 costs, in the terms of time and energy, to drill a hole in the prey shell compared to the direct entry
577 and consumption via the shell aperture. As suggested by our data (Test 2c), shell-drilling might be
578 the only way in which *Atopos* can complete the consumption of a *Plectostoma* snail with a fully-
579 grown shell. We did not find any signs of failed attempts of shell drilling (such as a scraped mark
580 without a hole, or a repaired hole). Nevertheless, some of the *Plectostoma* anti-predation traits,
581 namely, the tuba, the thickness of the shell wall, and the radial ribs could have played a role in
582 further increasing the predation cost to the shell-drilling predator.

583 In addition to the antipredation function towards preventing shell-apertural entry, the snail's tuba
584 also acts as a diversionary defence against shell-drilling. When a snail has withdrawn its soft
585 body into the spire, its tuba would be left vacant. We found evidence that the slug can be
586 deceived, as it were, to drill a (useless) hole in the tuba (this happens rarely, though: 3% of the
587 preyed shell in Test 1b, 8% - APO frequency in Table 1 of Schilthuizen et al., 2006). Moreover,

588 the slug would then drill a second hole in the spire (Test 1b) after the first drilling attempt at the
589 tuba. Finally, the low error rates in drilling suggests that *Atopos* individuals that frequently feed
590 on *Plectostoma* have learned (e.g. Kelley & Hansen, 2003) or their populations have evolved, to
591 distinguish the dummy tuba and the "edible" spire of the prey shell.

592 The penultimate line of defence against shell drilling, where shell traits are concerned, is the shell
593 thickness. We found that shell thickness is correlated with shell size (Test 1d, Figure 7). Although
594 we did not experimentally test the anti-predation role of shell thickness, we suggest that a thicker
595 shell may not fully protect the snail from shell-drilling by the slug, because we find drill holes on
596 the shells regardless of their shell thickness. Nevertheless, *Atopos* slugs probably need to spend
597 more energy and time to drill a hole through a thicker prey shell.

598 The *Plectostoma* snail's last line of defence is the rib intensity and rib density on the shell whorls.
599 We found that larger shells has low rib density (fewer ribs) than smaller shells, but the ribs of the
600 larger shells are more intense (longer and thicker) than the ribs of smaller shells. Despite the
601 variability in rib density, all of these snails are susceptible to drilling by the slug (Test 1b, Figure
602 5). Yet, *Atopos* avoids drilling through the more intense ribs on the less ribbed shells (Figure 5).

603 Nonetheless, we found a trade-off between rib intensity and rib density (see next section for more
604 discussion about this). Thus, a snail with a shell of higher rib density does not necessarily have an
605 anti-predation advantage over a snail with a shell of lower rib density. Although we do not know
606 if the slug would prefer prey that either have higher or lower rib density, the ribs on the prey shell
607 do impose a greater cost for the slug because it needs to drill through these ribs before the drill
608 hole breaches the shell wall. As suggested by Allmon, Nieh & Norris (1990), the sculpture of the
609 shell is not a very effective adaptation to resist predation by drilling. Others have suggested that
610 tall and strong ribs could make the shell effectively larger and therefore hinder the manipulation
611 by predator (Vermeij, 1977). These hypotheses still need to be tested in the *Atopos-Plectostoma*
612 interaction.

613 To sum up, *Plectostoma* anti-predation traits might mainly act to delay the predator, which
614 increases the time and energy requirement for *Atopos* to complete predation. The resistance
615 exhibited by the snail in response to shell-drilling by the slug cannot ensure the survival of the
616 preyed snail. Our results are in accordance with the general view that snail shells usually cannot
617 resist drilling by their predators (Vermeij, 1982).

618 **Why can't shell traits evolve to defend against both predatory strategies?**
619 *Atopos* has two effective predatory strategies to neutralise the defences of *Plectostoma* during the
620 last stage of predation. For both, it uses its digestive system (namely, its proboscis and digestive
621 fluid in the shell-apertural entry strategy, and its proboscis, radula and digestive fluid in shell-
622 drilling strategy). Thus, maintaining two predatory strategies that complement each other brings
623 no additional cost to the slug development. By contrast, *Plectostoma* has to invest in two different
624 sets of shell traits to deal with each of the predatory strategies. Yet, both sets of the shell traits
625 have orthogonal growth directions, which indicate a possible trade-off between the shell traits.

626 In a hypothetical situation where predators are present that attack only by shell-apertural entry,
627 snails can avoid predation by faster completion of a shell with tuba, which means the snail would
628 have to invest more resources (time and shell material) in the longitudinal growth of the shell. In
629 the alternative situation where predators are present that attack only by shell-drilling, snails can
630 avoid, or delay, predation by growing more thick flaring ribs, which means it would have to

invest more resources in the transverse growth and more frequent shifts from a longitudinal whorl growing mode to a transverse rib growing mode. Due to the orthogonal growth modes of these two shell traits, a snail cannot attain adult shell form faster when it needs to grow more ribs, and vice versa. This developmental trade-off causes the functional trade-off in the anti-predation traits of the shell. Therefore, none of the shell traits of *Plectostoma* are at an optimal level to defend against both shell-apertural entry and shell-drilling strategies of the *Atopos* slug.

Beside the trade-off between two set of shells traits, we also found a trade-off within one of the shell traits. From a theoretical point of view, the snail's shell could have evolved to have very dense, protruded and thick ribs to hinder *Atopos*'s drilling strategy. However, we found a trade-off such that ribs of more densely ribbed shells are less intense than ribs of the less densely ribbed shells. The underlying factors that cause this trade-off were not determined, but it does appear to reflect a developmental constraint.

To date, the majority of the antipredation adaptation studies have focused on the evolution of a single shell trait of the prey to a single predatory behaviour of one or more predators. However, in nature, a prey might possess several antipredation traits in response to several different predatory behaviours of a predator (e.g. Sih, Englund & Wooster, 1998; Relyea, 2003). Usually, a snail will counteract a particular predatory strategy with a single evolved anti-predation shell trait (Vermeij, 1993), but snails sometimes use a combination of more than one trait to defend against a predatory strategy (DeWitt, Sih & Hucko, 1999; Wada & Chiba, 2013). A few studies have shown that there may be a functional trade-off between such multiple anti-predation traits. For example, Hoso (2012) demonstrated that two snail anti-predation traitsevolved by changes in two different developmental mechanisms (shell coiling direction and foot structure) in response to two predation stages (capture and consumption) of the same predator. Here, we show another novel context of an anti-predation functional trade-off between two sets of anti-predation shell traits that are part of the same developmental mechanism (shell ontogeny), but in response to two different predatory behaviour at the same predation stages (consumption) by the same predator.

We found several correlations and trade-offs between and within the sets of anti-predation shell traits with each set having a specific function against a particular predatory strategy. However, more study is needed to clarify the exact causal relationships and to determine the underlying developmental biology of these shell anti-predatory traits. This could have important implications for our undrestanding of the evolutionary adaptability of shells under predation selection pressure in *Plectostoma* snail in particular and Gastropoda in general.

The co-evolution between *Atopos* predatory behaviours and *Plectostoma* anti-predation traits.

Predator–prey interaction has been one of the best-known examples of co-evolution between two species. In many cases, co-evolution between predator and prey can lead to evolutionary arm races, when both predator and prey continuously and reciprocally evolve improved predatory strategies and anti-predation traits while maintaining a stable ecological interaction; this is termed Red Queen evolution. In other cases, predation leads to unidirectional selection pressures impacting the evolution of the prey (Vermeij, 1987). As we have some empirical data of the predator-prey interaction between *Atopos* and *Plectostoma*, and have evaluated the costs and benefits of their predatory strategies and anti-predation traits, it is worthwhile to revisit the red-queen hypothesis that was proposed by Schilthuizen et al. (2006) for the evolutionary interaction between them.

675 Schilthuizen et al. (2006) examined drill hole patterns for 16 populations of *Plectostoma*to
676 establish possible links between the slug predatory behaviour and prey shell traits, which were
677 found by exploring the variation of slug predatory behaviour and snail traits among these
678 populations. They found that variation in predation behaviour was to some extent correlated with
679 variation in shell morphometrics (represented by principal component scores calculated from log-
680 transformed linear measurements of shells). Furthermore, variation of the shell morphometrics
681 was also correlated with the predation frequencies, which were estimated from the number of
682 empty shells with a drill hole as a proportion of the total number of empty shells.

683 In addition, Schilthuizen et al. (2006) also found two pairs of sympatric but morphologically
684 different *Plectostoma* populations, in which each member of the pairhad a similar pattern of drill
685 holes locations. Hence, they concluded that the slug drilling behaviour (i.e., preferred drilling
686 locations on the shell) was genetically determined and modulation by shell morphology. Finally,
687 they proposed that shell morphology of *Plectostoma* snails may evolve in Red Queen cycles with
688 co-evolving *Atopos* slug predatory behaviour.

689 Although our study was differently designed from Schilthuizen et al. (2006), our results may be
690 used to fill gaps in that previous study. The major gap was the fact that the mechanistics of the
691 interactions between the snail's antipredation traits and the slug's predatory behaviours were
692 unknown. In fact, data on the successes and failures, and the benefits and costs, of all the
693 predatory strategies and anti-predation traits are vital for the understanding on predator-prey
694 evolution (Vermeij, 1993). After critically analysing all the possible predatory strategies and
695 defensive traits, we found that the predatory path of the tuba, and the density and intensity of
696 shell ribs of the *Plectostoma* snails could have evolved under the shell-apertural and shell drilling
697 attacks by *Atopos*.

698 First, predatory path of the tuba was not included in Schilthuizen et al. (2006), but the density and
699 intensity of shell ribs was estimated from the maximum height of radial ribs and the numbers of
700 radial ribs per 0.5 mm on the penultimate whorl and tuba. We found that the slug tends not to drill
701 a hole through intense ribs (Test 1b, Figure 5). Hence, the diversity of drill hole location patterns
702 on the shell might be explained by rib density and intensity—a possibility that was not fully
703 considered in Schilthuizen et al. (2006). Large proportions of shells in the populations studied by
704 Schilthuizen et al. (2006) had drill holes on distinct locations, and these differed among
705 populations. For example, this was the case for theshell apex) of population GOMmir, and the
706 shell umbilicus of populations TABAco and TABAsi (Schilthuizen et al., 2006). We suggest these
707 drill hole locations could be due to the low rib intensity and density for these shell sectors in
708 these particular populations.

709 Second, the suggestion in Schilthuizen et al. (2006) that stereotyped slug drilling behaviour (in
710 terms of preferred drilling locations on the prey shell) is genetically determined needs
711 verification. As discussed above, the drill hole location might be influenced strongly by the rib
712 density and intensity. Thus, similar drill hole patterns in prey populations TABAco and TABAsi
713 could result from a non-genetic, behavioural response of the slug to the rib density and intensity
714 patterns on the prey shells. Further work is needed to determine the degree to which slug
715 behaviour may be a non-genetic behavioural response or a genetically determined adaptation to
716 prey shell traits.

717 Although our study could not reject the Red Queen evolution hypothesis, our results strongly
718 indicate that an alternative hypothesis should be considered: escalation of anti-predation traits in

719 *Plectostoma* populations as a response to a single, generalised set of predatory strategies in
720 *Atopos*. We showed that *Plectostoma* snails could have evolved a set of different anti-predation
721 shell traits, each of which has different efficiency against the slug's shell-aperture entry and shell-
722 drilling. Furthermore, we found that the slugs in most cases clear all the defenses and
723 successfully prey on the snail. However, the escalation hypothesis also needs to be tested in a
724 more comprehensive study, which should include more prey and predator populations in the area.

Conclusion

726 Our study has unravelled several aspects of the predator-prey interactions between the *Atopos*
727 slug and *Plectostoma* snails in the limestone habitats of Borneo. Despite having several distinct
728 anti-predation traits, such as protruding radial ribs and distorted coiling of the shell, *Plectostoma*
729 snails have low resistance against predation by the slug with its two predatory strategies (shell-
730 apertural entry and shell-drilling). The effectiveness of the snail's anti-predation traits is probably
731 limited by trade-offs imposed by ontogenetic constraints. Lastly, further experiments are needed
732 to test whether the evolution between *Atopos* slugs and *Plectostoma* snails is a case of either
733 escalation or Red Queen co-evolution.

Supporting Information

735 File S1. Raw data and supplementary information for results (Tables: S1 – S4, Figures: S1 –
736 S23).
737 File S2. Raw data and R script for data analysis for all tests.
738 File S3. Raw data for Test 2 (c): Effectiveness of prey shell whorl morphometrics against shell-
739 apertural entry by the *Atopos* proboscis.

Author Contributions

746 Conceived and designed the experiments: LTS. Performed the experiments: LTS. Analyzed the
747 data: LTS. Contributed reagents/materials/analysis tools: LTS MS. Wrote the paper: LTS MS.

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Figure 1

Flowchart shows experimental design for 12 research questions of this study.

Bold text represents the respective tests for each research question; text bounded in each diamond shape represents the predatory behaviour of *Atopos*; text bounded in each oval shape represents the Plectostoma shell trait that was tested for their anti-predation property.

1. What are the predators for *Plectostoma* species and what are their predatory strategies?
   - **Field observation**
   - **Test 1 (a)**
   - **Literature review**

Shell-drilling

Shell-apertural entry
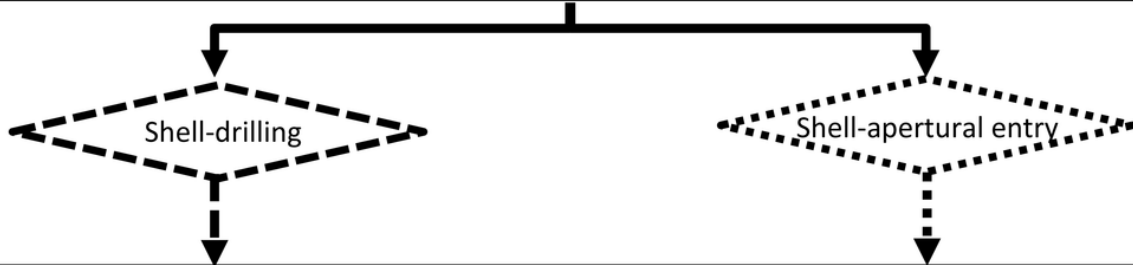
2. What are the possible anti-predatory traits of *Plectostoma concinnum* that could be inferred from predatory strategies of *Atopos* slug?
   - **Field observation**
   - **Test 1 (a)**
   - **Literature review**

Ribs on shell surface

Shell whorls thickness

Shell tuba

Animal resting behaviour
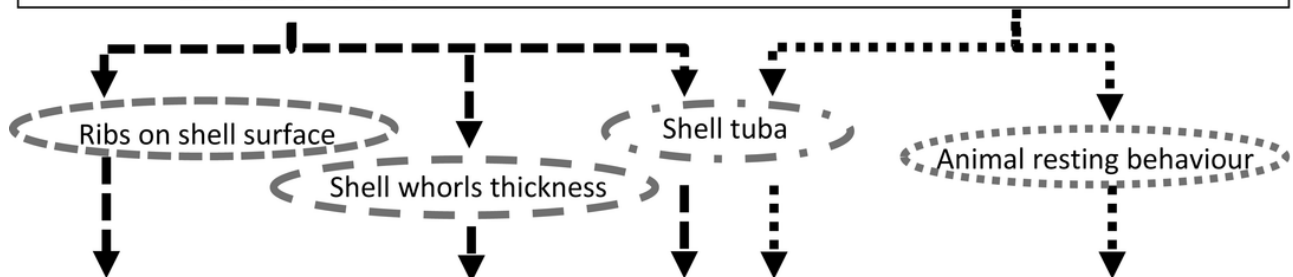
3. Is *Atopos* a stereotyped predator of *Plectostoma concinnum*?
   - **Test 1 (a)**

4. Does *Atopos* make characteristic drill holes on *Plectostoma concinnum* shells?
   - **Test 1 (a)**

5. Does *Atopos* drill holes on the tuba of the prey shell?
   - **Test 1 (b)**

6. Does *Atopos* have a tendency to drilling holes either on the ribs or between 2 ribs and does this depend on the rib density of the prey shell?
   - **Test 1 (b)**

7. Does the prey shell have higher rib intensity when it has higher rib density?
   Test 1 (c)

8. What is the variation of shell thickness in *Plectostoma* with respect to shell size and number of ribs?
   - **Test 1 (d)**

9. Does the predator prefer to attack particular prey shell forms, namely, juveniles without tuba, sub-adults with partial tuba, and adults with complete tuba?
   - **Test 2 (a)**

10. Can the slug remove *Plectostoma concinnum* snails from the substrate?
    - **Test 2 (b)**

11. Does the slug's ability in removing prey from the substrate depend on the size of the slug and the rib density?
    - **Test 2 (b)**

12. Could the slug's proboscis pass through the shell whorls via the aperture and reach the deeply withdrawn body in a *Plectostoma concinnum*?
    - **Test 2 (c)**

Figure 2

Predatory strategies that are used by *Atopos* slugs and Lampyridae beetle larvae to attack micro-land snails – *Plectostoma* species.

(A) *Pteroptyx* cf. *valida* (Olivier, 1883) larva, which was probably at its fifth instar, attacking *Plectostoma laidlawi* (Sykes, 1902) by shell- apertural entry. (B) *Pteroptyx tener* (Olivier, 1907) larva, which was probably at its fifth instar, attacking *Plectostoma fraternum* (Smith, 1905) by shell-apertural entry. (C) *Atopos* slug attacking *Plectostoma concinnum* (Fulton, 1901) by shell-drilling. (D) *Atopos* slug proboscis (marked with red outline) that was used for shell-drilling (the proboscis was not fully extended). (E) A drill hole on the shell of *Plectostoma concinnum* (Fulton, 1901) made by *Atopos*. (F) The appearance of the margin around the drill hole.
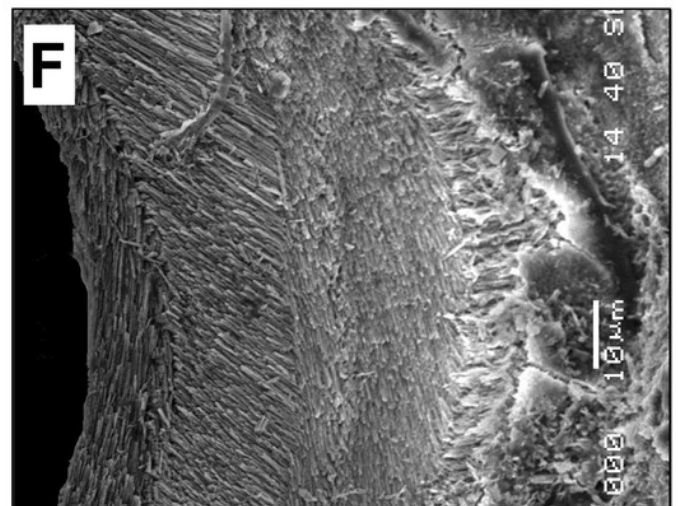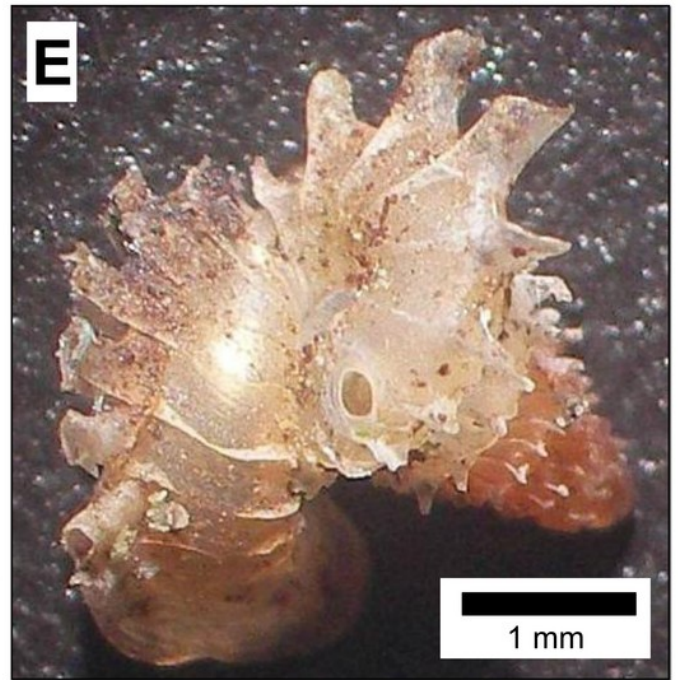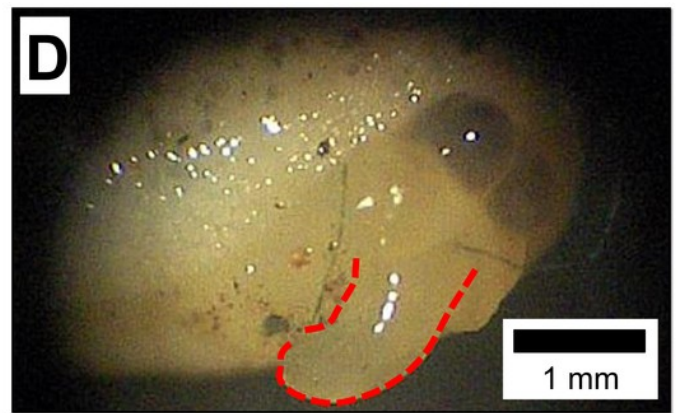
Figure 3

Figure 3. Procedures used to quantify the shell volume of material of the ribs and shell whorls (Test 1c).

Step (1) x-ray tomography by using high-resolution micro-CT scanner.

Step (2) Reconstruction of 2D cross-section binary images.
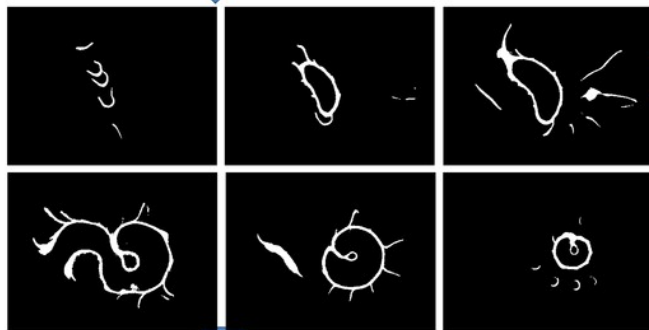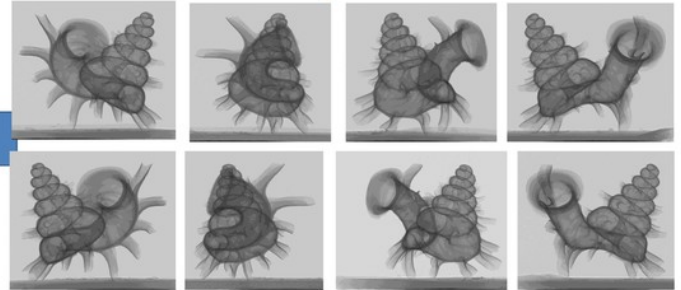
Step (4) Removing ribs from 2D cross-section binary images.
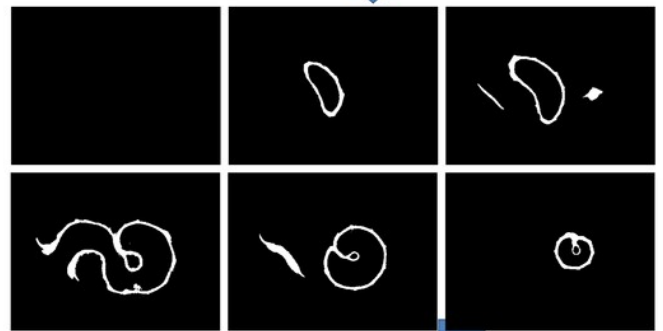
Step (3) Estimation of shell-material volume for whole shell.
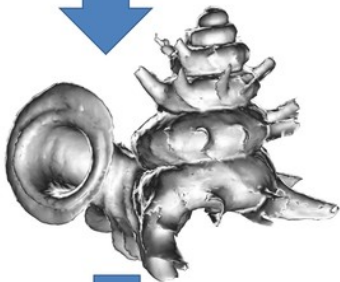
Step (5) Estimation of shell-material volume for shell-whorls only.

Step (6) Calculation of shell-material volume of shell ribs:
= Step (3) **minus** Step (5)

Figure 4

Shell withdrawal path analysis of *Plectostoma concinnum* (Fulton, 1901).

(A) Animal withdrawal depth at different growth stages of the shell. (B) Predatory path in the shell (red line). (C) Shell ontogeny axis (blue line). (D) Determination of animal withdrawal depth and growth stage by using photograph and 3D shell model. (E) Transferring information of predatory path and growth stage from each shell to an adult reference shell.

* Animal withdrawal depth

Growth stage – position at shell ontogeny axis of shell (a.)

Predatory path distance of shell (a.)
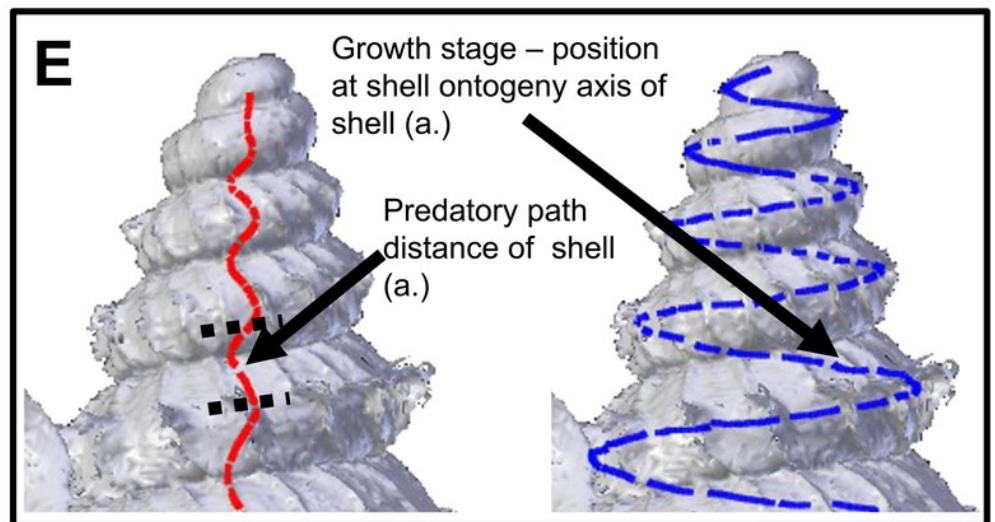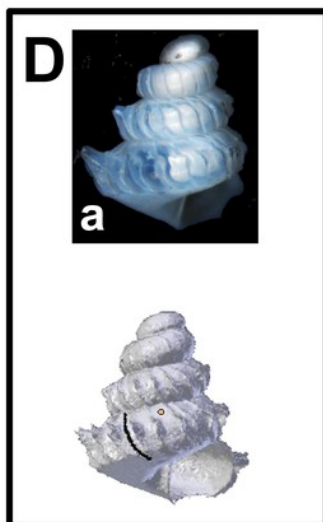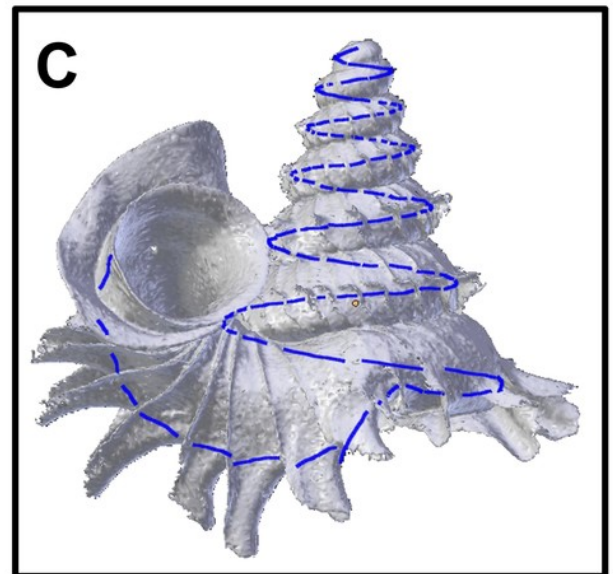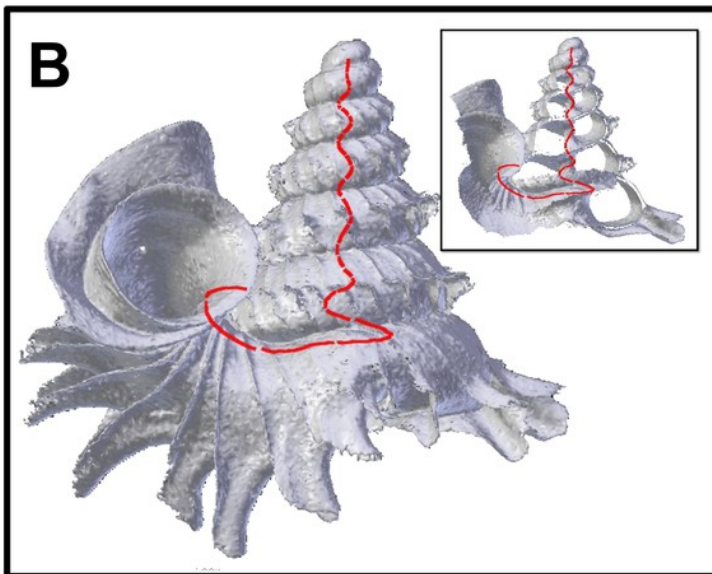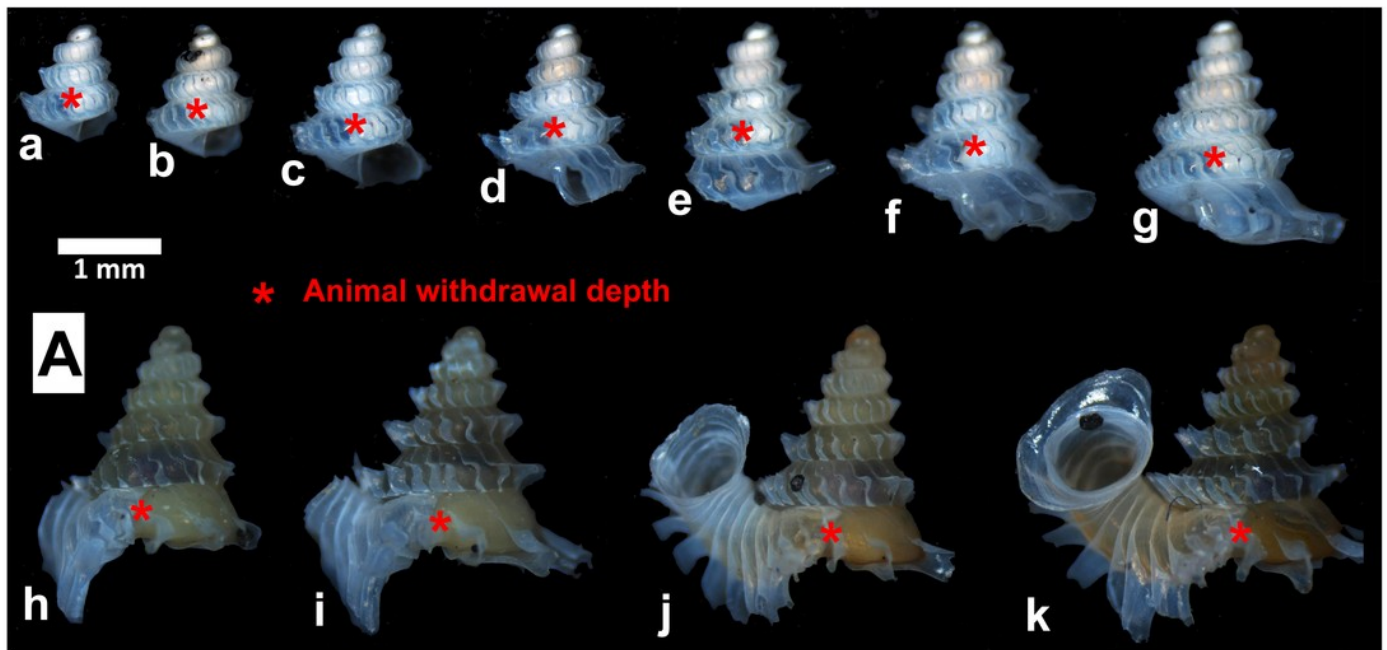
Figure 5

Analysis of the relationship between the likelihood of the slug drill hole BETWEEN RIBS and the three predictor variables.

(A) Proportion of the ribs spacings larger than HOLE SIZE for the shells (boxplot) and the proportion of shells having holes in between ribs (red asterisk) for each RIB DENSITY category. (B) – (D) Logistic curve showing the probability of the slug drill hole in between the ribs based on (B) RIB DENSITY (i.e., total number of ribs on shell), (C) HOLE SIZE (i.e., drill hole size, which represents the slug proboscis size), and (D) CHANCES (i.e., number of the ribs spacings that are larger than HOLE SIZE).
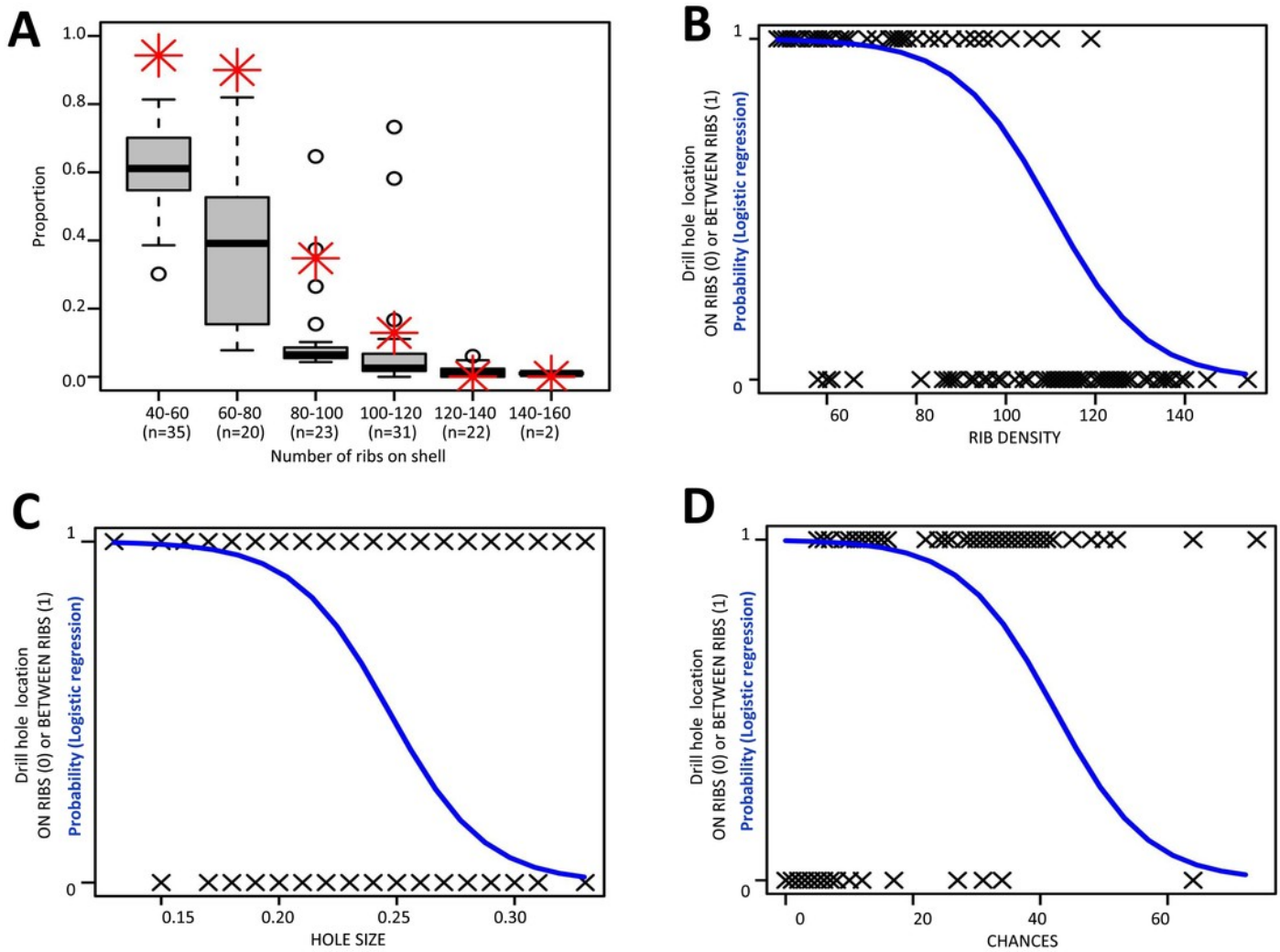
Figure 6

The graphs show the correlation between the number of ribs on the shell and rib intensity before and after controlling for shell size.

(A) Correlation between number of ribs on the shell and rib intensity (r = - 0.95, t = -10.74, df = 12, p < 0.001). The rib intensity (i.e. total shell material of all shell ribs in mm3 which belong to several *Plectostoma* species and populations that vary in rib number. The inset of four examples of shells. (B) The graph shows the partial correlation of number of ribs on the shell and rib intensity after correcting for total shell material volume (r = - 0.63, t = -2.71, df = 14, p < 0.001). The group mean values are represented by "0" on both axes.) and the number of ribs were measured from 14 shells,

A

Rib intensity (mm^3) vs Number of ribs on shell

$r = -0.95$, $t = -10.74$, $df = 12$, $p < 0.001$

B

Standardised residue of rib intensity (mm^3) vs Standardised residue of number of ribs

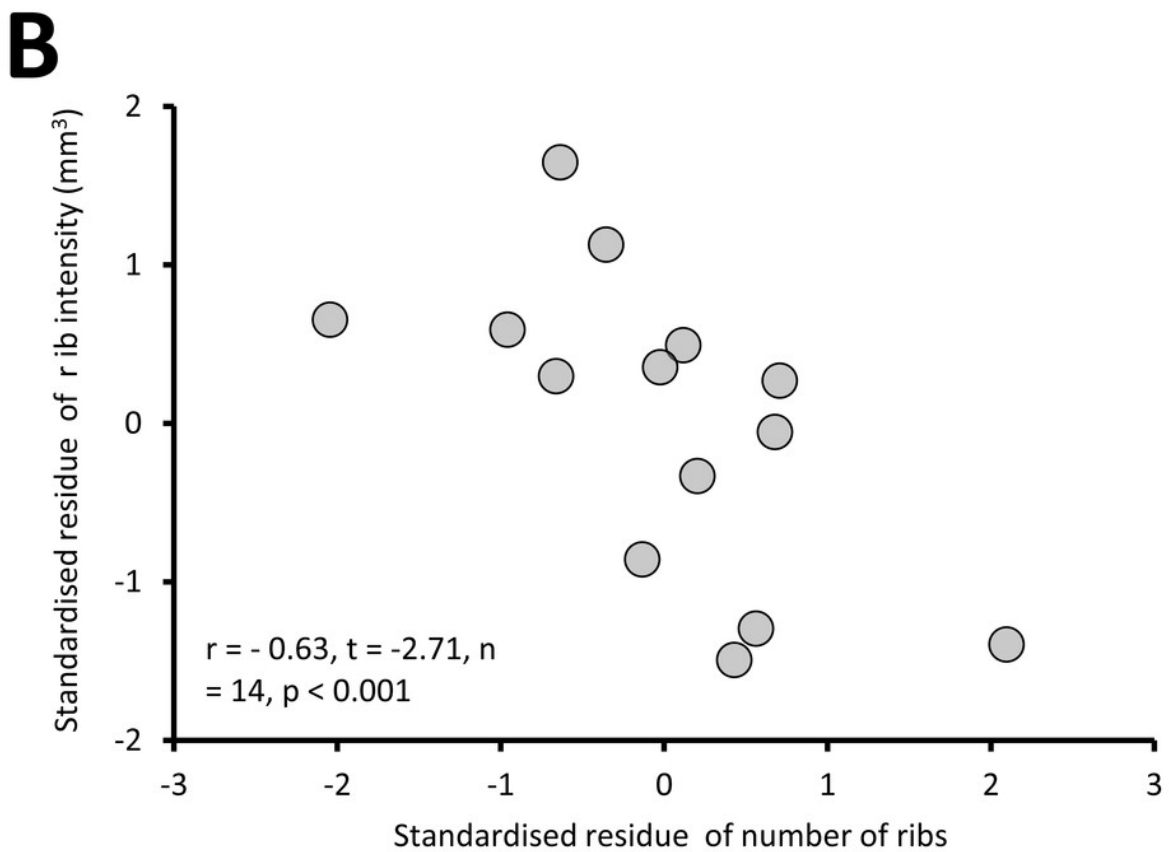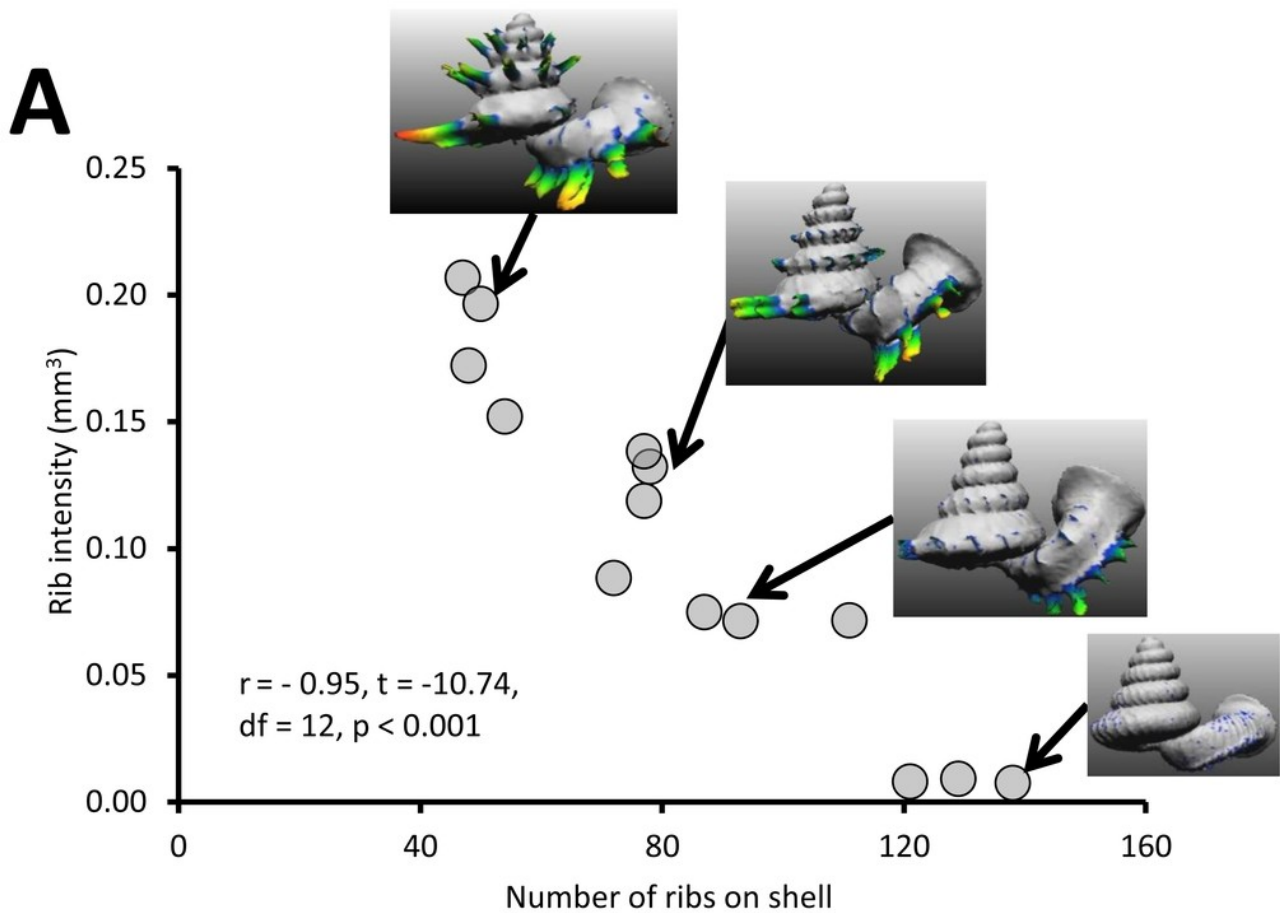$r = -0.63$, $t = -2.71$, $n = 14$, $p < 0.001$

Figure 7

The graphs show the correlation between the number of ribs on the shell and shell thickness before and after controlling for shell size.

(A) Correlation between the number of ribs on the shell and shell thickness (r = - 0.73, t = -3.7, df = 12, p < 0.005). The shell thickness (mm) was measured from 14 shells, which belong to several *Plectostoma* species and populations that vary in rib number. The inset of four examples of shells. (B) The graph shows the partial correlation of number of the ribs on the shell and shell thickness after correcting for total shell material volume (r = 0.06, t = 0.19, df = 14, p = 0.85). The group mean values are represented by "0" on both axes.

A
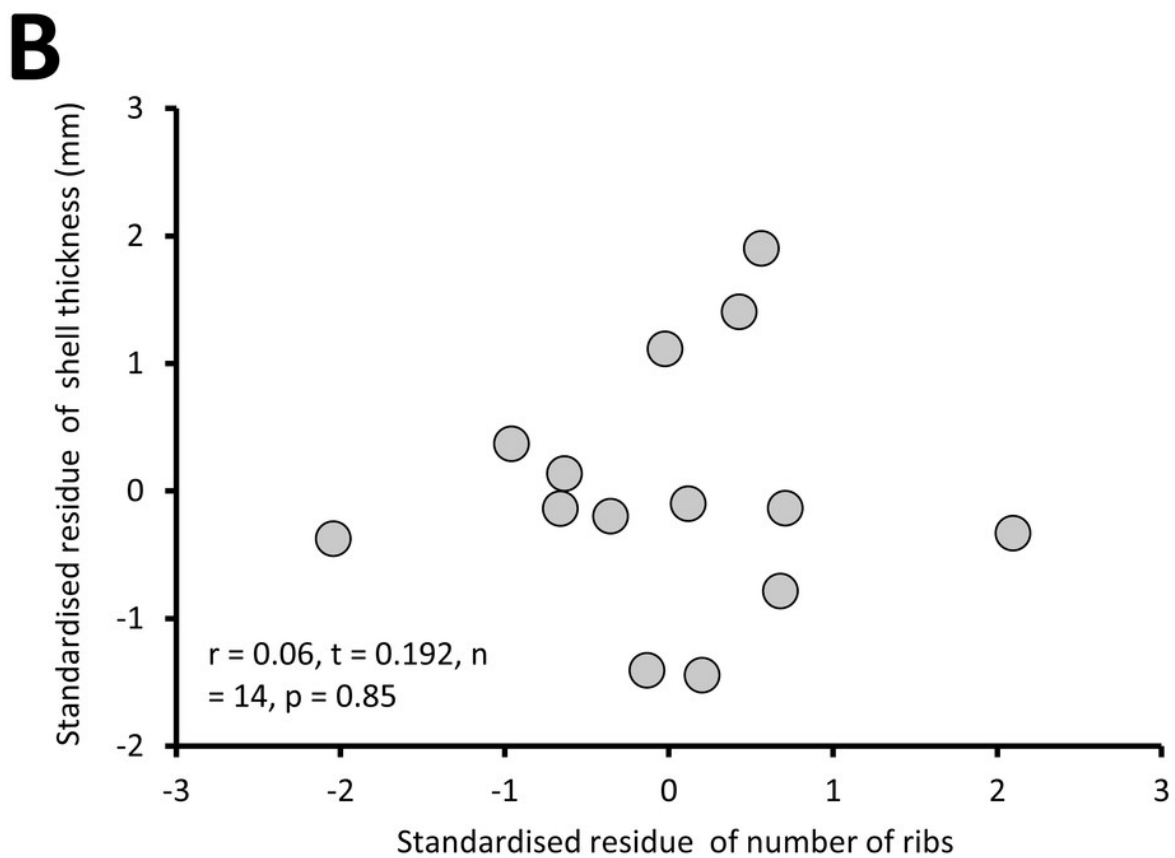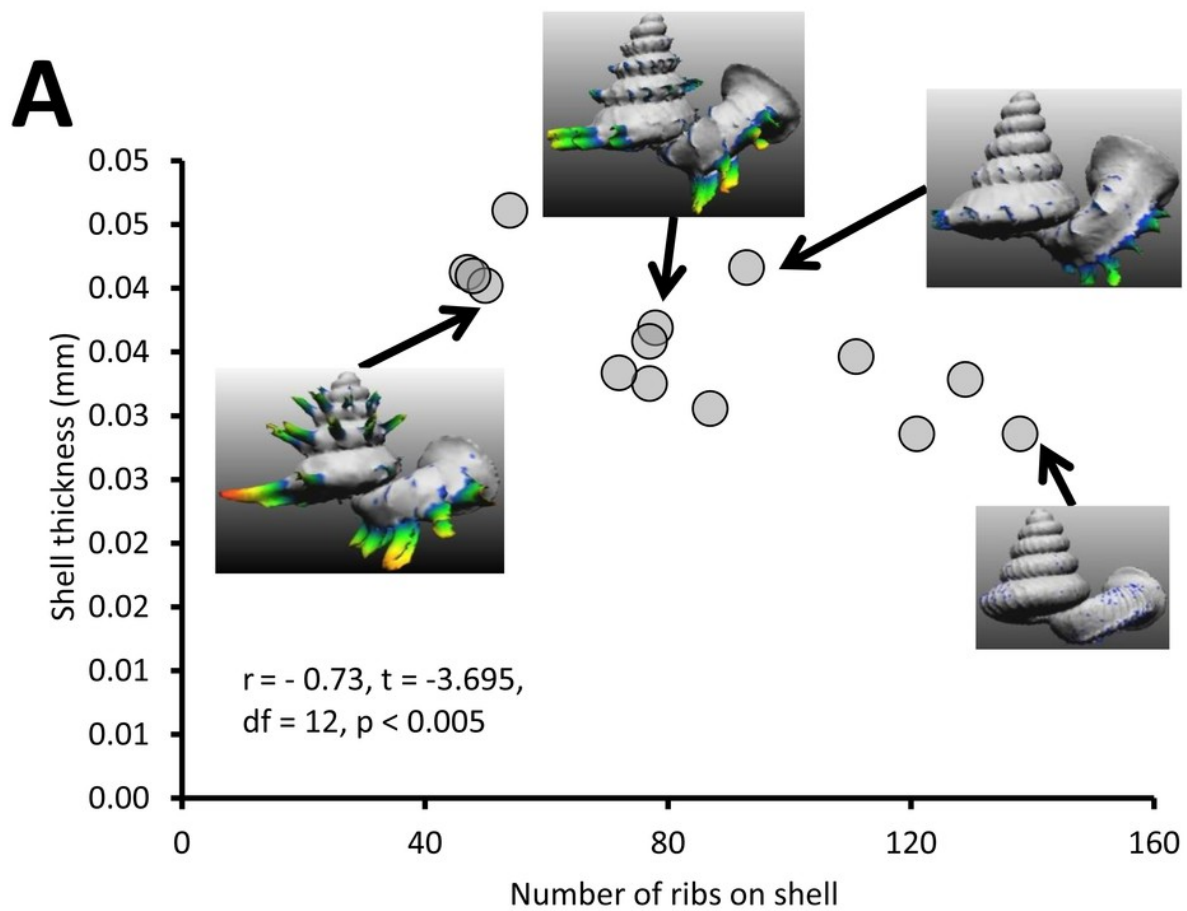
Shell thickness (mm)

0.05
0.05
0.04
0.04
0.04
0.03
0.03
0.03
0.02
0.02
0.01
0.01
0.00

r = - 0.73, t = -3.695,
df = 12, p < 0.005

0   40   80   120   160

Number of ribs on shell

B

Standardised residue of shell thickness (mm)

3

2

1

0

-1

-2

r = 0.06, t = 0.192, n
= 14, p = 0.85

-3   -2   -1   0   1   2   3

Standardised residue of number of ribs

Figure 8

Four examples of shell s after predation by apertural entry.

Each of them has an intact operculum that is attached to the posterior side of the shell aperture (arrows).
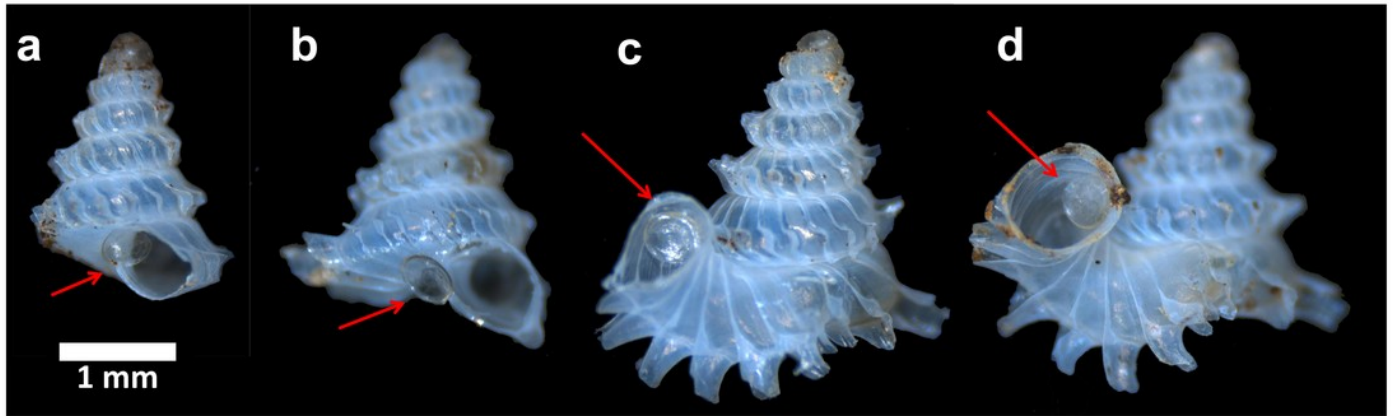
Figure 9

Analysis of the drill hole location on the shells.

(A) four different sectors of the shell whorls divided with reference to the snail's position when adhering to the substrate: Sector A – shell whorls facing the substrate; Sector B – shell whorls facing the tuba; Sector C – shell whorls at the back of Sector A; and Sector D – shell whorls at the back of Sector B. (B) Frequencies of drill holes found in each of four shell whorl sectors are significantly different ($\chi 2$ = 22.1, df = 3, p < 0.0001). (C) The rib density of the shells does not significantly differ among these four shell sectors (Kruskal-Wallis $\chi 2$ differ among these four shell sectors (Kruskal-Wallis $\chi 2$ = 3.71, df = 3, p = 0.29). (D) The drill hole size does not significantly = 7.17, df = 3, p = 0.06).
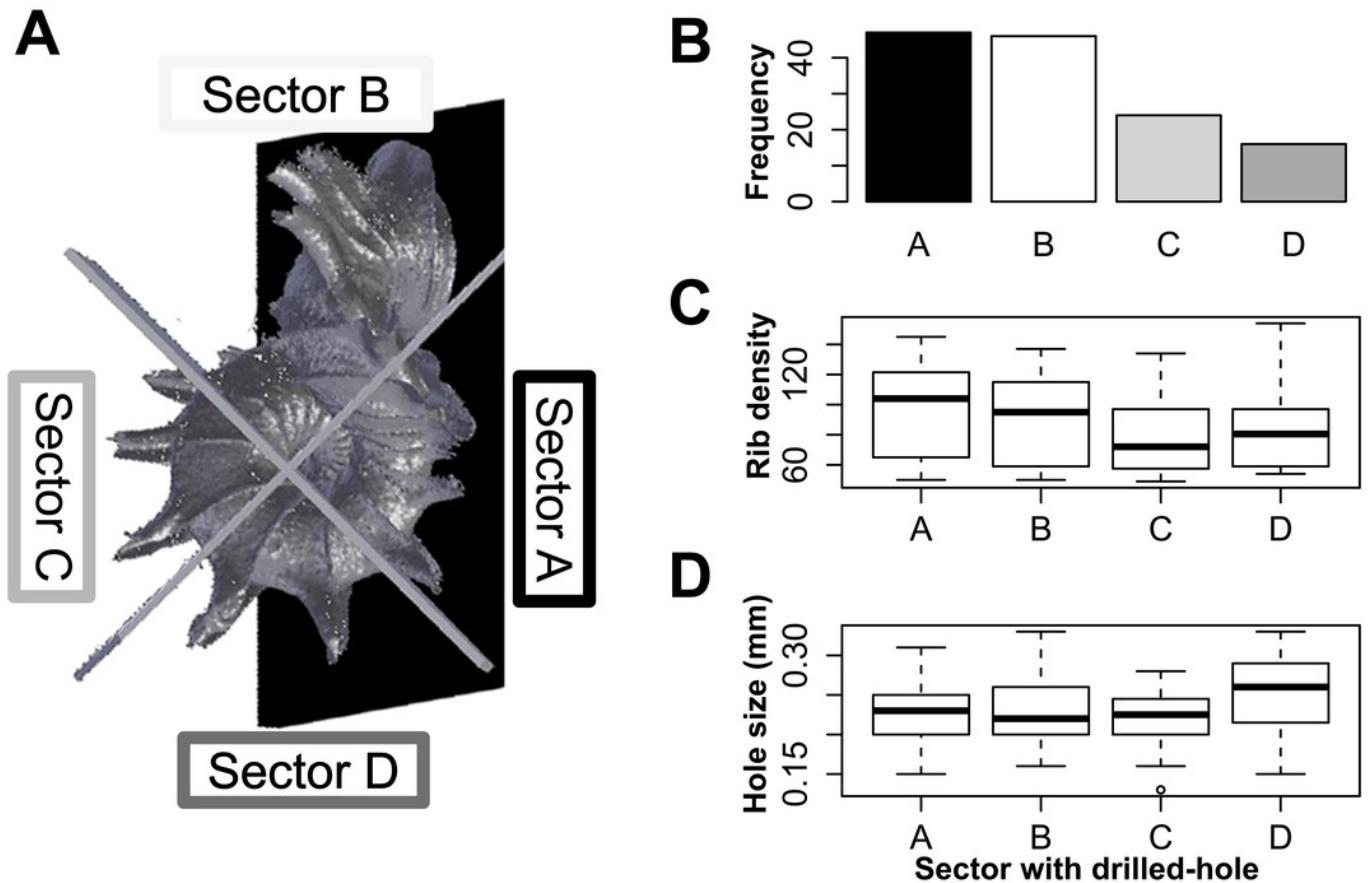
Figure 10

Association between the predator proboscis morphometrics (pink symbols) and the prey shell whorls morphometrics (black symbols).

Green boxes represent the section of shell ontogeny (i.e. prey growth stages) that are not susceptible to *Atopos* attack by shell-apertural entry (i.e. predatory path distance > proboscis length & whorl radius of curvature < proboscis diameter). The insets show the simulation of interaction between slug proboscis and snail predatory path at three growth stages, namely, a, f and l (see figure 3A). (A) Smallest predator scenario. (B) Largest predator scenario.
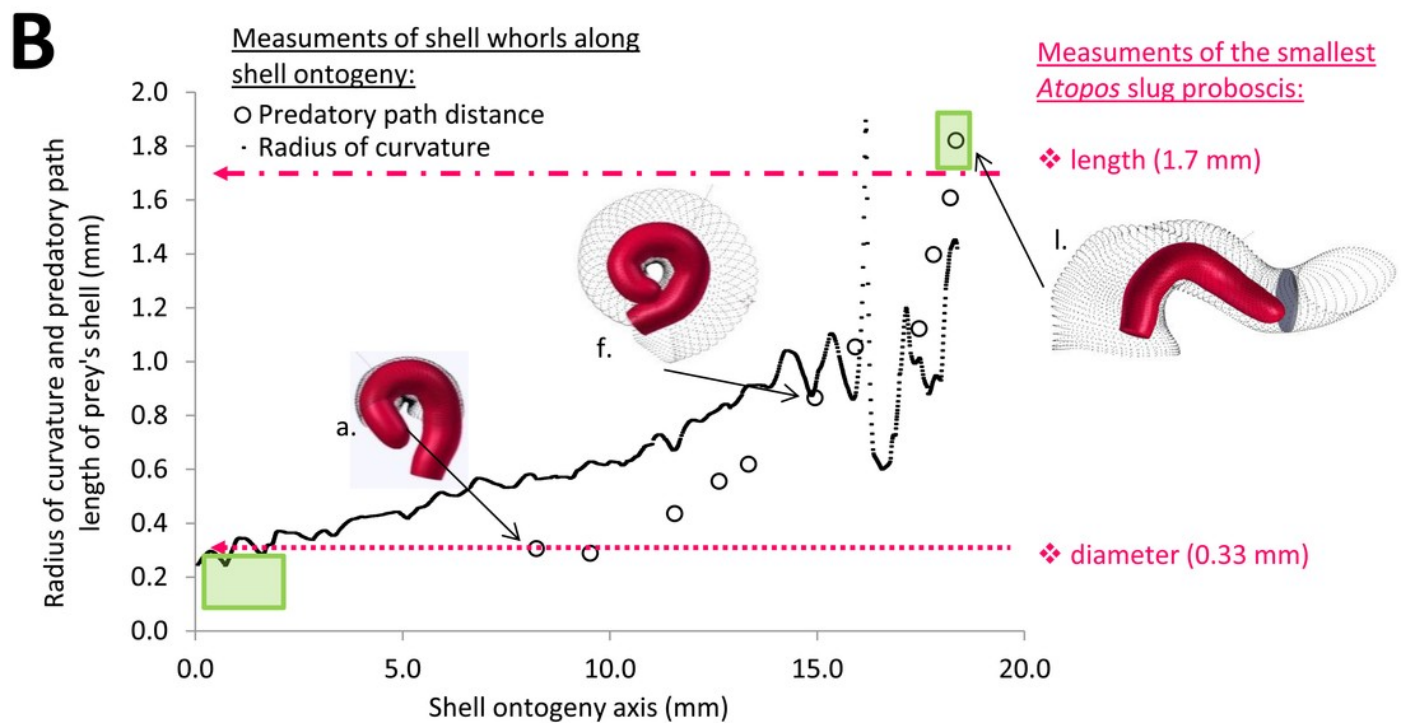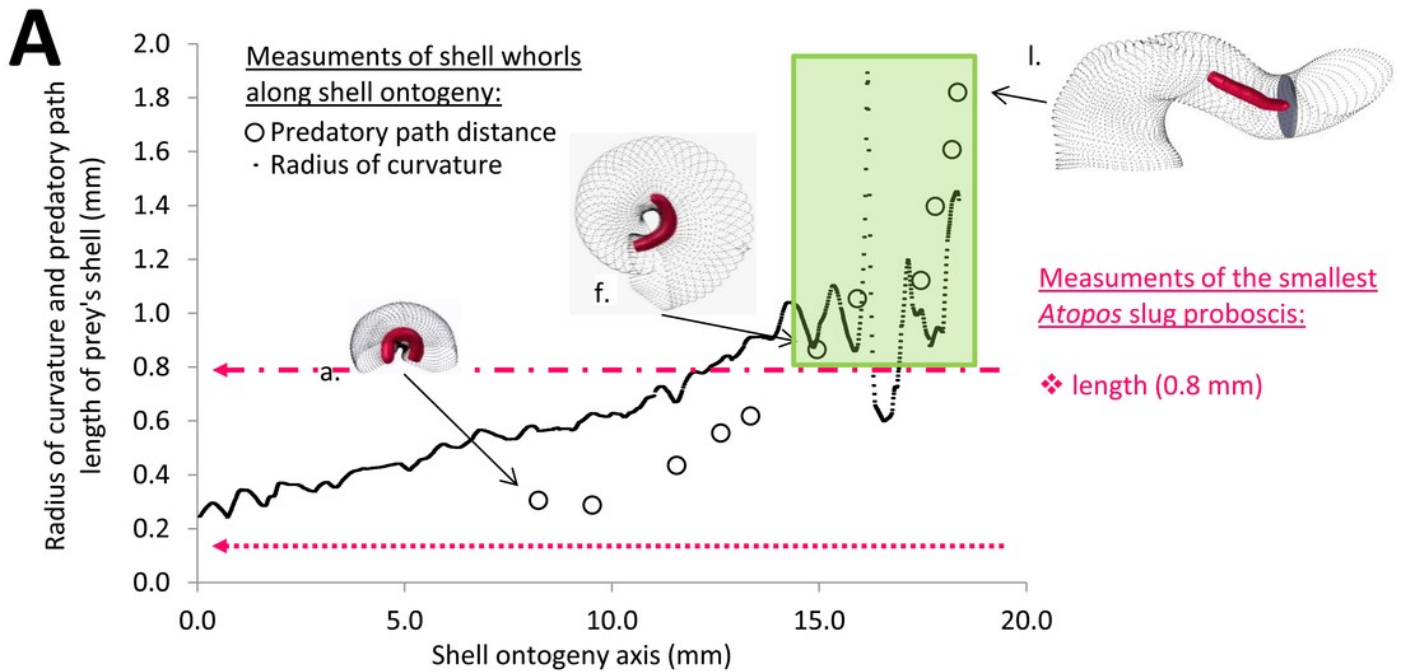
A

Measuments of shell whorls
along shell ontogeny:

○ Predatory path distance
· Radius of curvature

Radius of curvature and predatory path length of prey's shell (mm)

Shell ontogeny axis (mm)

I.

Measuments of the smallest *Atopos* slug proboscis:

❖ length (0.8 mm)

a.

f.

B

Measuments of shell whorls along shell ontogeny:

○ Predatory path distance
· Radius of curvature

Radius of curvature and predatory path length of prey's shell (mm)

Shell ontogeny axis (mm)

Measuments of the smallest *Atopos* slug proboscis:

❖ length (1.7 mm)

❖ diameter (0.33 mm)

I.

a.

f.

Figure 11

Shell whorl morphometric changes in torsion along the shell ontogeny.

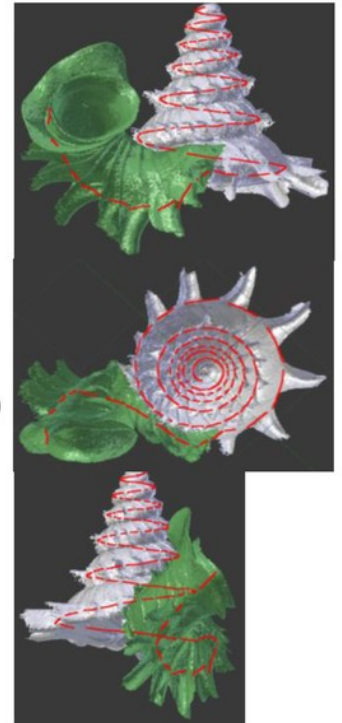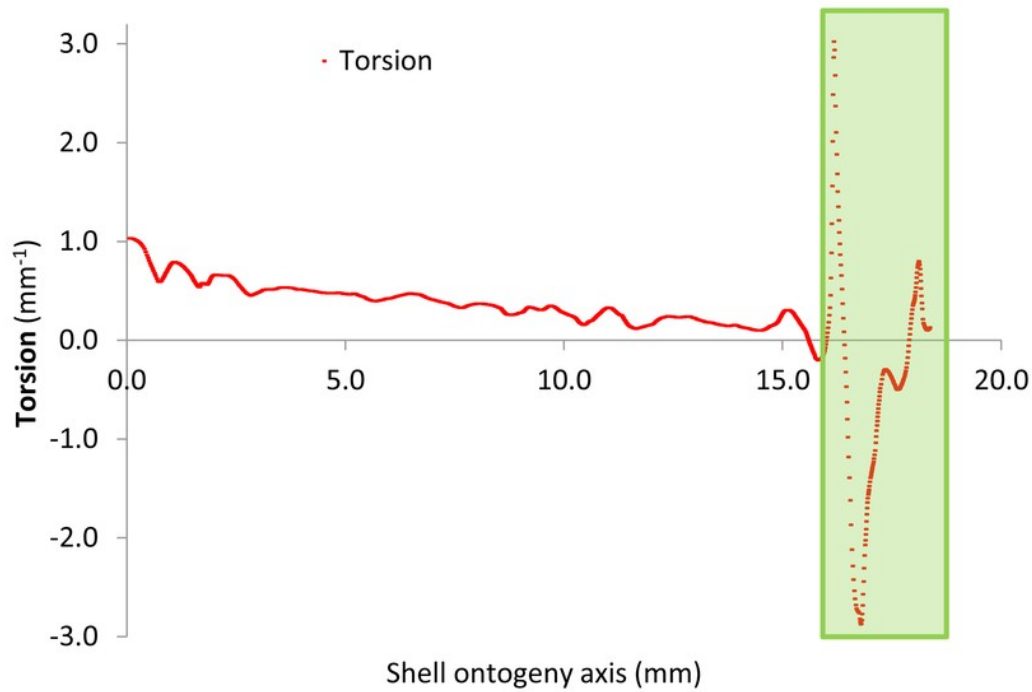The tuba part undergoes dramatic changes in torsion during the shell growth.

Table 1 (on next page)

Observation of predators and their predatory behaviour towards Plectostoma species in the field.

Table 1. Observation of predators and their predatory behaviour towards *Plectostoma* species in the field.

No. of occasion	Date and time	Location	Note	Observer
1	28th March 2003, Probably between 09:00 – 10:00 PM.	Malaysia, Sabah, Tomanggong Besar.	*Atopos* slug attacked *Plectostoma fraternum* (Schilthuizen et al. 2006). A total of 15 slugs were found within 25 m² og limestone rock face.	Menno Schilthuizen
2	9th May 2011, 11:30 AM	Malaysia, Sabah, Gomantong Cave.	*Pteroptyx tener* larva attacked *Plectostoma concinnum* (shell-apertural entry).	Liew Thor-Seng
3	9th May 2011, 11:34 AM	Malaysia, Sabah, Gomantong Cave.	*Pteroptyx tener* larva attacked *Plectostoma mirabile* (shell-apertural entry).	Liew Thor-Seng
4	28th May 2011, 10:25 AM	Malaysia, Kelantan, Kampung Bayu.	*Pteroptyx* cf. *valida* larva attacked *Plectostoma laidlawi* (shell-apertural entry).	Liew Thor-Seng
5	14th December 2011, 10:00 AM	Malaysia, Sabah, Batu Kampung.	*Atopos* slug attacked *Plectostoma concinnum* (shell-drilling).	Liew Thor-Seng & Mohd. Effendi Marzuki
6	20th January 2013, 09:15 AM	Malaysia, Sabah, Batu Kampung.	*Atopos* slug attacked *Plectostoma concinnum* (shell-drilling).	Liew Thor-Seng
7	18th January 2013, 10:30 AM	Malaysia, Sabah, Batu Kampung.	*Atopos* slug.	Liew Thor-Seng
8	21st January 2013, 08:25 AM	Malaysia, Sabah, Batu Kampung.	*Atopos* slug.	Liew Thor-Seng
9	18th January 2013, 10:15	Malaysia, Sabah, Batu	*Pteroptyx tener* larva.	Liew Thor-Seng

	AM	Kampung.		
10	20th January 2013, 08:40 AM	Malaysia, Sabah, Batu Kampung.	*Pteroptyx tener* larva.	Liew Thor-Seng

Table 2(on next page)

Literature survey of predatory behaviours of Lampyridae beetle larvae towards land snails.

Table 2. Literature survey of predatory behaviours of Lampyridae beetle larvae towards land snails.

Source	Predator	Habitat	Predatory strategy	Note
Madruga Rios and Hernández Quinta (2010)	*Alecton discoidalis* Laporte, 1833	Limestone forest, on left litter and on rock.	Shell–apertural entry.	- Preyed on 13 land snails species of 7 families. - Was found associated with operculate gastropods.
Clench and Jacobson (1968)	*Alecton* sp.	Limestone.	Shell–apertural entry.	- Land snail species *Viana regina* (Helicinidae).
Wang et al. (2007)	*Pyrocoelia pectoralis* (E. Oliv., 1883)	Grasslands and deserted farmlands.	Shell–apertural entry.	- Inserted their elongate heads together with their mouthparts into the shell to bite and chew at the snail bodies.
Archangelsky and Branham (1998)	*Pyractomena borealis* (Randall, 1838)	n.a.	Shell–apertural entry.	- Inserted their elongate heads together with their mouthparts into the shell to eat the content of retracted snail. - Injected extraoral disgesting fluids through their mandibles.
Thornton (1997:65)	lampyrid	Leaf litter.	n.a.	- Preyed on very small snail (2 mm), possibly *Gastrocopta pediculus*.

Table 3(on next page)

Literature survey of predatory behaviours of Rathouisiidae slugs towards land snails.

Table 3. Literature survey of predatory behaviours of Rathouisiidae slugs towards land snails.

Source	Predator	Habitat	Predatory strategy	Note
Heude (1882-1890)	*Rathouisia leonina* Heude, 1882	Wet and shaded brick wall cracks of the building.	Shell–apertural entry.	- Could eat up to two small prey and stay up to three days without food.
Wu et al. (2006)	*Rathouisia leonina* Heude, 1882	Limestone hills and urban garden, humid and shady habitat.	Shell–apertural entry and shell-drilling.	- Inserted its head or its proboscis alone through aperture depending upon aperture size of the prey. - Fed on eggs by drilling a hole. - The size of bored hole is related to the size of the predator.
Tan and Chan (2009)	*Atopos* sp.		Shell–apertural entry.	- Held the prey with the anterior part of the foot arched in a straddling position. - Inserted its proboscis into the prey shell via aperture.
Kurozumi (1985)	*Incillaria* sp.	Around limestone rocks, wet part of the forest.	Shell–apertural entry and shell-drilling.	- Fed on eggs by drilling a hole. - There was a narrow scraped part on the margin of the hole. - Drilled hole on the shell of prosobranch snails such as *Georissa fukudai* and *Cyclophoris turgidus*. - Attacked other snails through aperture.

Table 4(on next page)

Data from Test 2 (a) – Predation behaviour in relation to prey shell morphology.

Table 4. Data from Test 2 (a) – Predation behaviour in relation to prey shell morphology.

No.	*Atopos* slug ID	Observation starting time	Estimated starting and ending time of the predation by slug.	Duration (Hour: Minutes)	Snail survivorship of each shell form category*		
					Adult	Sub-adult	Juvenile
1	No. 7 of Table 1.	22:04, 18/01/2013	14:00 - 18:30, 19/01/2013	4:30	S	P**	P
3	No. 8 of Table 1.	11:50, 20/01/2013	22:00, 20/01 - 06:00, 21/01	8:00	S	p	S
5	No. 8 of Table 1.	06:30, 21/01/2013	13:00, 21/01 - 22:20:00, 21/01	9:20	S	p	p
7	No. 8 of Table 1.	22:22, 21/01/2013	22:22, 21/01/2013 - 06:45, 22/01/2013	9:07	S	p	p
8	No. 8 of Table 1.	06:45, 22/01/2013	21:50, 22/01/2013 - 05:30, 23/01/2013	9:20	S	p	p
9	No. 8 of Table 1.	05:30, 23/01/2013	15:00 - 18:00, 23/01/2013	3:00	S	p	Missing***
10	No. 8 of Table 1.	18:15, 23/01/2013	18:15, 23/01/2013- 10:55, 24/01/2013	16:40	S	p	p
11	No. 8 of Table 1.	11:00, 24/01/2013	18:15, 24/01/2013- 09:00, 25/01/2013	14:45	S	p	S
12	No. 8 of Table 1.	09:00, 25/01/2013	23:00, 25/01/2013 - 06:00, 25/01/2013	7:00	S	p	p

* "S" – snail survived after experiment, "P" – snail was preyed by *Atopos* slug in the experiment.
** Half of the animal was consumed
*** Specimen was lost during the handling and thus the status of survival of this individual was unknown.