




Article

The Taming of *Smeagol*? A New Population and an Assessment of the Known Population of the Critically Endangered Pulmonate Gastropod *Smeagol hilaris* (Heterobranchia, Otinidae)

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Abstract: The genus *Smeagol* consists of five named species of air-breathing marine slugs (restricted to southern Australia and New Zealand) and three undescribed taxa from southern Japan. Only one species, *S. hilaris*, is known to be from New South Wales (NSW), and it previously had a known distribution limited to one site, Merry Beach on the south coast. This diminutive invertebrate is classified as critically endangered in NSW due to its extremely restricted distribution and concern about its historically declining numbers. Accordingly, the aims of this study were to survey the known population of *S. hilaris* at Merry Beach and to explore other potentially suitable sites, using a visual census method, to determine if further populations or species exist in NSW. The resulting quantitative surveys of the known population and a new population at Storm Bay, Kiama, NSW, are reported here. DNA barcoding of a ~650 bp segment of the mitochondrial cytochrome *c* oxidase I (COI) gene for several individuals from each population confirmed the conspecificity among the two populations. The population at Merry Beach was found to remain viable, while the discovery of the new population of *S. hilaris* represents a doubling of the known global populations of this species. Details of the highly-specialised niche habitat occupied by *Smeagol* in New South Wales and recommendations for ongoing management are documented.

Keywords: threatened species; visual census; conservation management; boulder beach fauna; Smeagolinae; gravel maggots



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1. Introduction

The gastropod genus *Smeagol* Climo, 1980, contains five named species [1,2], all of which are confined to southern Australia and New Zealand, and a set of three undescribed species from southern Japan [3]. Three of the named species are found in south-eastern Australia: *Smeagol phillipensis* Tillier & Ponder, 1992, *S. parvula* Tillier & Ponder, 1992, and *S. hilaris* Tillier & Ponder, 1992. Although *S. parvula* has been found on both sides of Bass Strait at Phillip Island, Victoria, and Devonport, Tasmania, *S. phillipensis* and *S. hilaris* exhibit highly restricted distributions, with both known only from their type localities [1]. All species of *Smeagol* are found in intertidal boulder habitats [1,3–5], where they live under cobbles and rocks. As air breathers, they are found in the mid to upper intertidal zone in freely draining cobbles set among gravel [1]. Little is known of their biology,

with most of the literature associated with taxonomic details or analyses of gastropod phylogeny [1,6–10].

Only one species is known to occur in New South Wales (NSW), *S. hilaris*, which previously had a known distribution limited to a few narrow gravel and boulder filled gutters at Merry Beach on the NSW south coast. *Smeagol hilaris* is classified as critically endangered in NSW due to its extremely restricted distribution, concern about declining numbers (Ponder and G. Haszprunar collected 25 specimens in 1987, but were only able to locate 10 in 1991 [1]), and vulnerability to threatening processes, e.g., habitat damage, invasive species, and the effects of climate change [11,12]. With only a few recorded observations, the undescribed Japanese *Smeagol* species are also classified as threatened [13–15].

As a critically endangered species, priority management actions include regular monitoring of the known population, in addition to surveys of potentially suitable habitats elsewhere [12]. In accordance with this, the aim of this study was to (1) survey the known population of *S. hilaris* at Merry Beach and to (2) explore other potentially suitable habitats using a visual census method while minimising habitat disturbance. As a result, a quantitative survey of the known population and a new population at Storm Bay, Kiama, NSW is reported here. Given that the two allopatric Victorian species, *S. phillipensis* and *S. parvula*, are separated by only a few kilometres of coast, the conspecificity of these two populations was examined using the barcode mitochondrial cytochrome *c* oxidase 1 (COI) gene [7,16–18]. Details of biotic and abiotic features of the *Smeagol* habitat are also provided.

2. Materials and Methods

2.1. Species Identification

Smeagol is recognised by its distinctive appearance: it is a limaciform intertidal slug with a translucent white body. The brown anterior digestive gland and yellow to orange posterior gonad are visible through the transparent notum. Although the Australian species are generally separated by morphological differences [1], *S. hilaris* may be delimited from its congeners by its size (5–6 mm) and location.

2.2. Survey Sites

Several boulder beaches on the NSW south coast were surveyed for *Smeagol*: Bass Point, Shellharbour (34°35'55.27" S 150°53'56.46" E); Little Blowhole Beach, Kiama (34°41'16.22" S 150°51'59.33" E); Loves Bay, Kiama (34°41'51.09" S 150°51'44.01" E); Storm Bay, Kiama (34°40'19.92" S, 150°51'30.95" E); O'Hara Head, Kioloa (35°33'36.33" S 150°23'39.18" E); Pretty Beach, Kioloa (35°34'16.01" S 150°22'17.42" E); Pebbly Beach, Kioloa (35°35'44.16" S 150°19'45.03" E); Shelly Beach, Kioloa (35°32'43.24" S 150°22'55.03" E), and Merry Beach, Kioloa (35°33'41.47" S, 150°22'44.57" E). Of these, only Merry Beach and Storm Bay (Figure 1A,B) were found to shelter *Smeagol*.

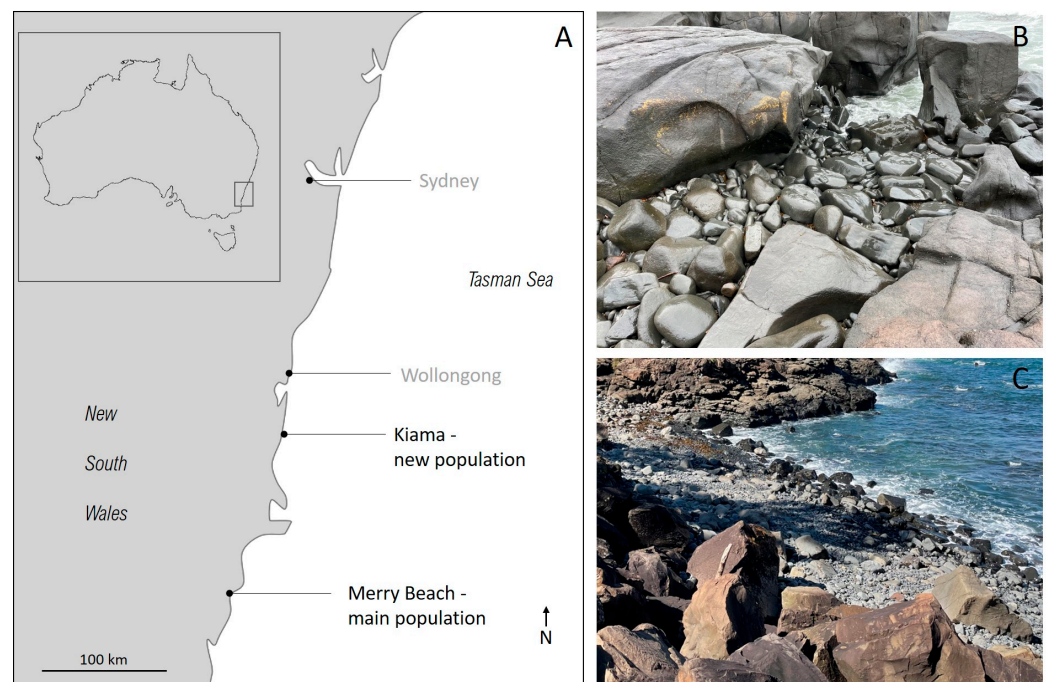


Figure 1. (A) Locations of *Smeagol hilaris* populations in south-eastern NSW: (B) survey site at Merry Beach; (C) survey site at Storm Bay.

2.3. Specimen Collection

Specimens—five from Merry Beach and four from Kiama—were collected for further study. These were relaxed in magnesium sulphate (7.5%) and preserved in ethanol (95%). The specimens were deposited with the Australian Museum, Sydney (AMS) (Table 1). The specimens were collected under DPI Scientific Collection Permit P01/0059(A)-4.0.

Table 1. Details of *Smeagol* specimens and sequences used in this study.

Species/AMS Accession	Crawl Length (mm)	Location	Isolate	GenBank Accession	Collector
<i>S. hilaris</i> AM C.594555.1	6.0	Storm Bay, Kiama	Kiama1	OP950367	MN
<i>S. hilaris</i> AM C.594555.2	5.5	Storm Bay, Kiama	Kiama2	OP950366	MN
<i>S. hilaris</i> AM C.594555.1	6.0	Storm Bay, Kiama	Kiama3	OP950365	MN
<i>S. hilaris</i> AM C.594556.1	6.0	Storm Bay, Kiama	Kiama4	OP950364	MN
<i>S. hilaris</i> AM C.594556.2	5.5	Merry Beach, Kioloa	Merry1	OP950363	LH
<i>S. hilaris</i> AM C.594556.3	6.0	Merry Beach, Kioloa	Merry2	OP950362	LH
<i>S. hilaris</i> AM C.594557.1	5.5	Merry Beach, Kioloa	Merry3	OP950361	LH
<i>S. hilaris</i> AM C.594557.2	6.0	Merry Beach, Kioloa			LH
<i>S. hilaris</i> AM C.594557.3	6.0	Merry Beach, Kioloa			LH
<i>S. phillipensis</i>	-	Phillip Island, Victoria		FJ917283	-
<i>Ophicardelus ornatus</i>	-	-	-	DQ093530	-
<i>Otina ovata</i>	-	-	-	EF489389	-

2.4. Survey Methods

The extent of the cobble gutter at Merry Beach was measured in metres (L × W). A search transect was plotted out which comprised a 2 m wide by 5 m long band centered on the neap high water mark (determined by the deposition of wrack) that ran parallel to the shore. Within the search transect, an estimate of population size was determined by counting individuals within two small areas (0.4 m² and 0.25 m²) to minimise disturbance. These areas were searched by hand, with cobbles excavated individually to a depth of approx. 20 to 30 cm. The undersides of the cobbles were examined for *Smeagol*, with any animals found photographed and returned to the habitat. Searches were also conducted above and below the designated search band to determine whether *Smeagol* was present outside the designated search transect. The steep boulder beach at Storm Bay was initially searched to determine the vertical distribution of *Smeagol* on the beach face. Surveys were then carried out as per Merry Beach. At this site, an estimate of population size was determined using three randomly distributed 1 m² quadrats, situated at the elevation where *Smeagol* were located. The vertical distribution limits of *Smeagol* were quantified using a builder's level from a nearby fixed survey mark.

2.5. Molecular Methods

DNA was extracted from ~10 mg of tissue from each specimen using a modified low-salt CTAB protocol (MolSC) following the protocol developed by Arseneau, Steeves, and Laflamme [19]. The quality of the extracted DNA was checked using Nanodrop, Qubit, and 2% agarose gel electrophoresis assays. Partial COI sequences were amplified using degenerate primers [20] (jgLCO1490 5'- TITCIACIAAYCAYAARGAYATTGG -3', jgHCO2198 5'- TAIACYTCIGGRTGICCRARAAYCA -3'). A master mix was prepared using 10.0 µL of Invitrogen Platinum II Hot-start PCR Master mix (2×) PCR buffer, 0.4 µL of each primer (10 uM concentration), 8.2 µL H₂O, and 1 µL of extracted DNA for each reaction. Polymerase chain reaction (PCR) cycling was carried out using an Eppendorf Nexus thermal cycler with the following conditions: initial denaturation at 95 °C for 3 min, 8 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s, with another 32 cycles at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 45 s. Finally, elongation was carried out at 72 °C for 5 min. After amplification, the presence of a PCR product was confirmed by means of 2% agarose gel electrophoresis. The amplicons were outsourced for purification and sequencing at the Australian Genomic Research Facility (AGRF), Sydney.

Bi-directional sequence reads were de novo assembled using Geneious 11.1.5 [21] and edited by eye. Barcode marker sequence data for *Smeagol phillipensis*, as well as *Ophicardelus ornatus* and *Otina ovata* (as outgroups following Dinapoli and Klusman-Kolb [8]) were retrieved from GenBank (Table 1), and all sequences were then aligned using the MAFFT plugin [22] using default settings. The primers were trimmed from the alignment. Data quality checks were carried out with MEGABLAST [23] and protein translation.

Conspicuity was tested using COI sequence data. Within- and between-putative species mean distances (p-distances) were calculated using MEGA V7.0.26 [24]. A phylogenetic tree based on the MAFFT alignment was produced by W-IQ-Tree [25,26] using default settings. W-IQ-Tree incorporates ModelTest [27], which selected the HKY model as the best-fit model, scored according to Bayesian information criterion (BIC), which was automatically applied. The resultant tree was visualised using FigTree 1.4.4 and rooted using *Ophicardelus ornatus*.

3. Results

3.1. Merry Beach Population

At Merry Beach, survey efforts were concentrated on the largest of three gravel gutters, the only place with accessible boulder, cobble, and gravel habitat at the time of survey (due to unfavourable conditions, other cobble gutters were not searched).

A total of 37 individuals (ranging between 3 to 6 mm crawl length, Figure 2A) were observed at Merry Beach on 7 April 2022. These individuals were found under rocks and small boulders that were set within gravel (Figure 2B). All specimens were found along the 2 m wide search transect along the neap high tide line, with no individuals detected above or below this transect.

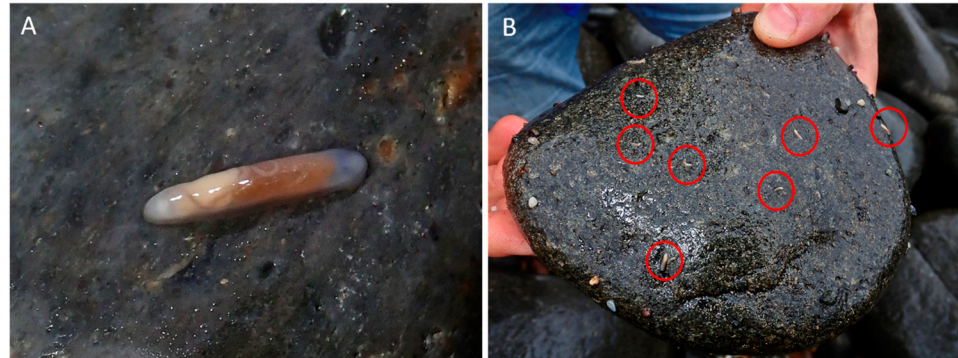


Figure 2. *Smeagol hilaris* at Merry Beach, NSW, 7 April 2022: (A) adult specimen; (B) eight individuals on a single cobble (circled in red). Photos: Tom R. Davis.

At Merry Beach, 27 individuals were found in a 0.4 m² area and another 10 in an area of 0.25 m²; this translates to an average density of 59.2 per m². Therefore, within the estimated habitat area of 10 m², there is the potential for approximately 592 individuals. It should be noted, however, that the habitat was not homogenous, being interspersed with large boulders which effectively reduced the available habitat area, potentially reducing the projected population. However, as *Smeagol* are generally found under cobbles to a depth of 30 cm, the abundance estimate obtained from a shallow examination of surface rocks alone may also be conservative.

3.2. Storm Bay, Kiama, Population

Storm Bay at Kiama (Figure 1C) is a narrow, steeply sloping boulder beach, situated between two rocky headlands surrounded by parkland in close proximity to the town centre. On 15 April 2022, a total of 31 individuals were observed at Storm Bay, Kiama, with three specimens collected (Figure 3A,B). All animals were found in the north-eastern part of the beach within an area 5.3 × 22.5 m (119.25 m²) and were restricted to 0.78 to 1.95 m above mean sea level in the beach profile (above the vertical distribution limit of the shelled gastropod *Hinea braziliiana* (Lamarck, 1822)). The total number of individuals among the three quadrats used for density estimates was 13; thus, there is an estimated population of 515 individuals across the suitable habitat area. As for Merry Beach, *Smeagol* were only found on the under surface of cobbles and boulders within coarse gravel.

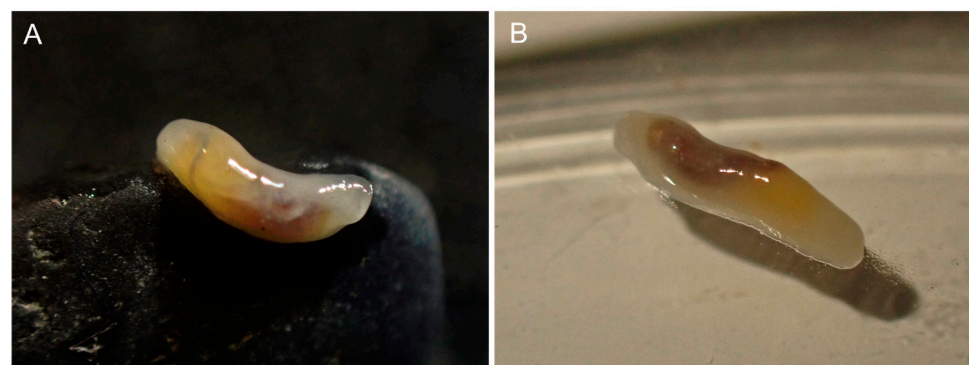


Figure 3. *Smeagol hilaris* at Storm Bay, Kiama, NSW, 15 April 2022 (A) in situ; (B) specimen lodged with the Australian Museum (AM C.594555). Photos: Matt J. Nimbs.

3.3. Molecular Analysis

PCR amplification and sequencing yielded 658 bp of COI. MegaBLAST searches of the NCBI database identified the best matches for all novel sequences generated by this study to COI sequence data of *Smeagol phillipensis*, (GenBank accession FJ917283.1).

The phylogenetic reconstruction based on maximum likelihood recovered a single species, with sequences from specimens from Storm Bay, Kiama, clustering among sequences from *S. hilaris* from Merry Beach, collectively forming a single clade (Figure 4). The mean within group distance based on uncorrected *p*-distances was 0.012 for *S. hilaris* from Merry Beach and 0.008 for *Smeagol* from Storm Bay, Kiama, whereas the mean between-group distance for the two populations was 0.007. Thus, as the genetic divergence between the two populations was less than the genetic divergence within each of the two populations, conspecificity was supported (Table 2). As only one partial sequence of *S. phillipensis* (~550 bp) was available from GenBank, it was not possible to calculate intra-specific distances for this species; however, it exhibited a higher between-group *p* distance (0.013) than that between the two *S. hilaris* groups, confirming its status as a separate taxon.

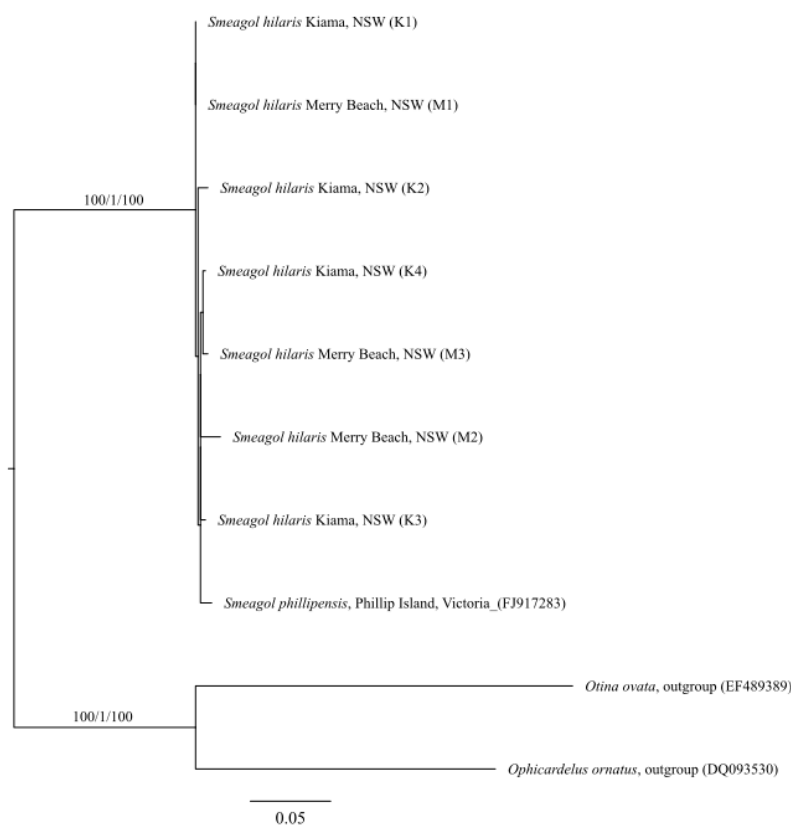


Figure 4. Molecular phylogeny of *Smeagol hilaris* from Merry Beach and Kiama, NSW, rooted with *Ophicardelus ornatus* and *Otina ovata*. The topology and branch lengths come from the maximum likelihood (ML) analysis of the COI sequences. Branch support values are approximate Bayes values (left), ML bootstrap (centre), and SH-aLRT support (right).

Table 2. Mean between- and within-species distances using uncorrected *p*-distances. Between-group distances are in bold.

	Distance between and within Species		
	<i>S. hilaris</i> Merry Beach, NSW	<i>S. sp.</i> Kiama, NSW	<i>S. phillipensis</i>
<i>S. hilaris</i> Merry Beach, NSW	0.012		
<i>S. sp.</i> Kiama, NSW	0.007	0.008	
<i>S. phillipensis</i>	0.010	0.013	N/A

3.4. Habitat Characteristics

The sites where *S. hilaris* occurred differed from the other sites. The Merry Beach site is a steeply sloping basalt rock platform with several deep gutters that run perpendicular to the shore. In the gutter where *S. hilaris* was found, there is a deep accumulation of basalt talus in the form of large boulders and rocks packed with cobbles and gravel, free of sand (Figure 5A). Algal wrack was held among the rocks at the neap high tide level (7.5 m from the westernmost part of the gutter) (Figure 5B). Cobbles and rocks within this algal wrack zone were found to yield the most individuals. The neap high tide line within this gutter has a span of 5 metres, and all *S. hilaris* were found within one metre of this line. Hence, the available area of the surveyed habitat at Merry Beach was calculated to be approximately 10 m², notwithstanding a large solid boulder that sits within this band, effectively reducing the available area (Figure 5A).



Figure 5. *Smeagol* habitat at Merry Beach, NSW. (A) Boulder- and cobble-filled gutter in a basalt rock platform; the measuring tape spans the gutter at the neap high tide line; (B) boulders, cobbles, gravel, and algal wrack at the neap high tide line. Photos: Matt J. Nimbs.

The Storm Bay, Kiama, site is a south-east facing, steep, basalt boulder and cobble beach with a shore length of 77 m. The beach is bound on both sides by rocky headlands. *Smeagol* were found under rocks that were located beneath kelp wrack at the north-eastern end of the beach (Figure 6).

The sites where *S. hilaris* were not found lacked either the deep, cobble-filled gutters, a steep beach face gradient, or the large quantities of wrack at the neap high tide mark. As these sites contained material ranging from coarse sand to large (>500 mm) boulders, they exhibited many of the general beach characteristics found at Merry Beach and Storm Bay.



Figure 6. *Smeagol* habitat at Storm Bay, Kiama, NSW. (A) Greywacke boulder and cobble beach; (B) algal wrack at the neap high tide line (*Smeagol* were found under rocks beneath wrack material). Photos: Matt J. Nimbs.

4. Discussion

The relatively high number of individuals found at the Merry Beach site, compared to previous surveys [1], indicates that the population of *S. hilaris* continues to be viable at this site. This finding, along with the discovery of an additional population at Kiama, confirmed by molecular tests, are highly important outcomes for this species.

The abiotic conditions in the *S. hilaris* habitat can be extreme, with high insolation and large swells, which can cause physical disturbance and saltwater inundation. In the week prior to the surveys, large swells were recorded on the NSW south coast, which appear to have removed many of the smaller cobbles and pebbles previously found at the Merry Beach site. Despite this, *S. hilaris* survived, most likely due to an ability to migrate deep within the gutter to avoid wave-induced strong hydrodynamic forces and the mechanical grind of tumbling rocks near the surface. Furthermore, the under-surface of large rocks, where *S. hilaris* resides, would have provided refuge from solar radiation and prevented prolonged freshwater and saltwater immersion through the provision of air pockets and free drainage (Figure 7).

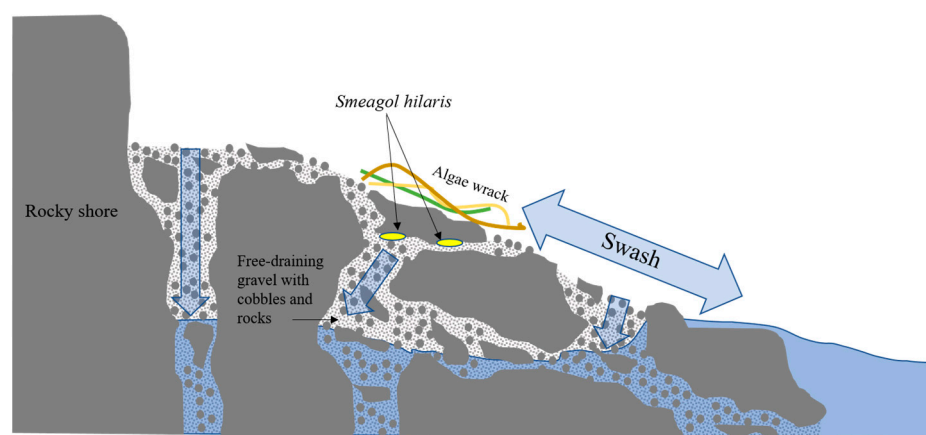


Figure 7. *Smeagol hilaris* habitat conceptual diagram. Animals are found beneath cobbles and rocks embedded in the upper 20–30 cm layer of gravel in freely-draining steep rock and boulder beaches at the neap high tide mark where algal wrack is deposited by swash.

In comparison to rocks and boulders found in the lower intertidal zone, which often support highly diverse sessile organisms [5], the under-surfaces of cobbles and rocks in the *Smeagol* habitat were found to support a relatively low diversity of macrobiota, most likely due to infrequent immersion and occasional mechanical disturbance. Other motile macroinvertebrates found sharing the undersides of cobbles at *Smeagol* sites (some of which, at first glance, may be mistaken for *Smeagol*) were: an unidentified white polyclad flatworm; Diptera; Coelopidae larvae (maggots); and the shelled gastropod, *Hinea brasiliana*. The former and latter, in addition to species of ellobiid snails in the genera *Leuconopsis* and *Marinula* were also reported by Tillier and Ponder as present at the Merry Beach site [1].

The discovery of a new population 140 km north of Merry Beach highlights the importance of conducting further surveys of potentially suitable habitats elsewhere on the southern NSW coast (particularly the coast south of Batemans Bay, NSW), with other similar boulder and cobble beaches known to occur in this region (T. Daly, pers. commun.). The Storm Bay, Kiama, population is located close to a seasonal tourist destination, and the proximity to this urban area may impact the ongoing viability of *S. hilaris* at this site; however, the specific impacts are not yet clearly understood. Here, the population may be impacted by runoff from terrestrial sources (pesticides, freshwater, oil, etc.), heavy foot traffic, and beach manipulation; therefore, habitat protection measures are recommended in order to mitigate these and other potential threats that urbanisation may pose. Perturbations such as oil spills can also profoundly reduce available habitat, as has occurred for two other species of *Smeagol* off Phillip Island in Victoria, Australia [28].

The high variability among historic estimates of individuals of *S. hilaris* at Merry Beach, along with the estimate from the current study, highlight the importance of conducting ongoing monitoring of population variations to determine whether the fluctuations are natural or are being driven by anthropogenic effects. Furthermore, there is a clear need for improved understanding of the species' biological and reproductive processes in order to assist with its management and identify potential threats to its ongoing survival. Research into this area may inform potential breeding and stocking programs, fill knowledge gaps, and refine targeted risk-abatement strategies.

There is no public awareness of *S. hilaris* at the Kiama site. An education campaign targeted at key stakeholders, such as the Kiama Council, nearby local residents, and tourists may help raise the profile of *S. hilaris* and create public support for the conservation of populations at both locations. The distribution of advisory material, potential public signage, and interpretive displays should be considered to inform the public about this highly vulnerable species.

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Conflicts of Interest: The authors declare no conflict of interest.

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