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Appendix 1. Error-rates in reclassification of dermis- and spine-colours of urchins

Methods

One hundred adult (>4cm diameter) sea urchins were collected from east Killarney, Warrnambool Breakwater and Port Phillip Bay between 9 August 2006 and 17 August 2006. Samples were collected from different locations and habitats in Victoria in an attempt to observe a range of colour variation in *H. erythrogramma.* Urchins collections were brought to shore where colour was recorded since low light and turbidity in the water may hinder classification accuracy (Growns 1991). A digital photo was then taken of each urchin against a white dive slate background. To compensate for light exposure the automatic mode was used. Urchin dermis and spine colour variation in the photos were observed to determine whether the classification scheme devised by Growns (1991) could be applied. Photographs of urchins were then used to define the classification scheme (see Appendix 2 for details of rules used to distinguish colours and examples of urchin photographs).

Experiment 1

To test the robustness of the classification scheme, the 100 urchin photos were classified by H. Beck using the classification scheme based on Growns (1991). Fifty photos, haphazardly selected by another person, were then reclassified by H. Beck. This was done five times, conducting each classification session (50 different photographs) five days apart to prevent photo memorisation by Beck. The misclassification (i.e. classified as a colour different to the initial classification) rate each time was calculated by dividing the number of different reclassifications by total classifications ×100. Dermis and spine colour classifications were treated separately.

Experiment 2

To determine whether urchin colour can be accurately classified by someone else, the 100 photos were shuffled and three different people independently scored the colour variation using the classification scheme. This was repeated five times for each person (one classification of 50 photos every second day to prevent memorisation) and the misclassification rate was calculated for each person, for each classification run.

Experiment 3

To determine whether colour classification in the field was different from when colour was classified in photographs, 200 photographs of urchins (their colour was classified in the field before having their picture taken) were randomly selected and their colour later reclassified by viewing digital photos on a computer screen. A large number of photos was chosen to ensure that this would include photographs taken under different light conditions including bright sunny days and dim, late afternoon conditions.

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Results

Most urchins that were collected could be allocated to the defined colour categories with relative ease. Dermis colour could be classified readily as white or dark red for the majority of urchins. Pink dermis urchins were found, but were rare. In contrast, spine colour varied considerably. Violet, green, violet to green, and white spines were found. Spine colour intensity also varied and ranged from very faint to very dark. For violet–green spined urchins, violet always occurred at the base and the amount ranged from a fine ring to extending half way up the spine. In some cases, it was hard to differentiate between white dermis, white spine (WW) and white dermis, green spine (WG) urchins. Moreover, where the urchin dermis was red, it was also difficult to distinguish green spines from violet green spines. Brown spines were also found, but this was rare. Photos of representative urchins were selected to define the colour range for each of these colour categories (*see* below).

Experiment 1

The mean misclassification rate of dermis colour classification was 1.2% (±1.79 s.d.), whereas the mean spine colour misclassification rate was 2.8 % (±1.79 s.d.). Of the 100 urchins reclassified across 250 classifications, either spine or dermis colour was different only seven times. Different classification mostly occurred where dermis colour was pink, whilst spine classification was mostly different for red dermis urchins where it was difficult to differentiate between green and violet–green spines.

Experiment 2

No significant difference was found in the rate of dermis colour misclassification among people and the overall 95% confidence interval for dermis colour classification error was between 0.09 and 2.45% (Table A1, Fig. A1). However, there was a significant difference in the number of wrong classifications of spine colour made between people (Table A2, Fig. A1).

Experiment 3

After 200 randomly selected urchin photographs, spine and dermis colour classification from digital photos was the same as that made in the field for 191 urchins. Hence, only 4.5% of reclassifications were different.

Table A1. ANOVA for the dermis colour classification error rate among three different people

Source	d.f.	Mean square	*F*	*P*
Person	2	0.867	0.788	0.477
Error	12	1.100		

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Table A2. ANOVA for spine colour classification error rate among three different people

Source	d.f.	Mean square	*F*	*P*
Person	2	11.667	11.290	0.002
Error	12	1.033		

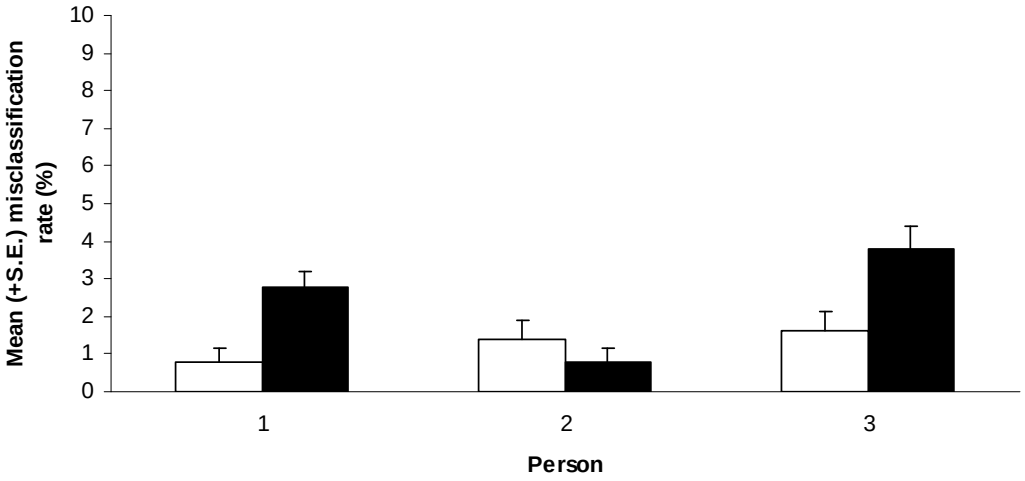

Fig. A1. Mean (+S.E.) misclassification rates for dermis (white bars) and spine (black bars) colour for three people after five trials of classifying 50 randomly selected urchin photographs.

Discussion

Obviously, since this study was prior to the main field surveys and colour was only analysed in three locations, the full extent of the possible colour morphs of *H. erythrogramma* across its range was not observed in these pilot experiments. However, most urchins here could easily be allocated to the classification categories defined by Growns (1991). In the cases where urchin spine or dermis colour was difficult to categorise, rules were made that were also applied in the main study. Where it was difficult to determine if their spines were white or pale green, the spines were scored green if they had a green tinge. When it was difficult to determine if spines were green or violet-green on red dermis urchins, they were scored as violet-green. Despite these complications, classification error rates were low.

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Dermis classification had a misclassification rate of 1.2 (±1.78) % for the classifier (H. Beck) used in the main study, whilst spines were classified differently by him 1.6 (±1.67) % of the time. These low error rates suggest that the classification system was a repeatable way of quantifying colour in *H. erythrogramma* and should be capable of detecting even small differences in the frequency of colour between samples. Furthermore, dermis colour classifications could be made by people without prior classification experience. While there were detectable differences among people in their consistency of spine colour classification, error rates were low for all of them anyway. Furthermore, the use of photographs appears to be an accurate way to record and directly compare urchin colour among samples. Consistency in urchin colour classification between those made in the field and from photographs suggests that digital photography is an accurate way to compare samples collected at different times and from different places. From the 200 reclassifications conducted, colour was reclassified from photographs differently in only <5% of samples. Since the photographs reclassified were taken on a number of days and under quite different lighting conditions that ranged from bright sunny days to dim overcast days, this suggests light should not affect the accuracy of classifying urchin colour from photographs.

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Appendix 2. Dermis and spine colour classification scheme

Dermis colour classification

(a)

(b)

(c)

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Fig. A2. These urchins are representative of (*a*) white, (*b*) pink and (*c*) red dermis colour categories.

(*a*) (*b*)

Fig. A3. These urchins are representative of (*a*) light brown and (*b*) white/brown dermis colour categories. Urchins with a light brown dermis have no red tinge or white colouration between spine bases. Urchins with a white/brown dermis had brown skin at the base of the spine, but white skin between spine bases.

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Primary spine colour classification

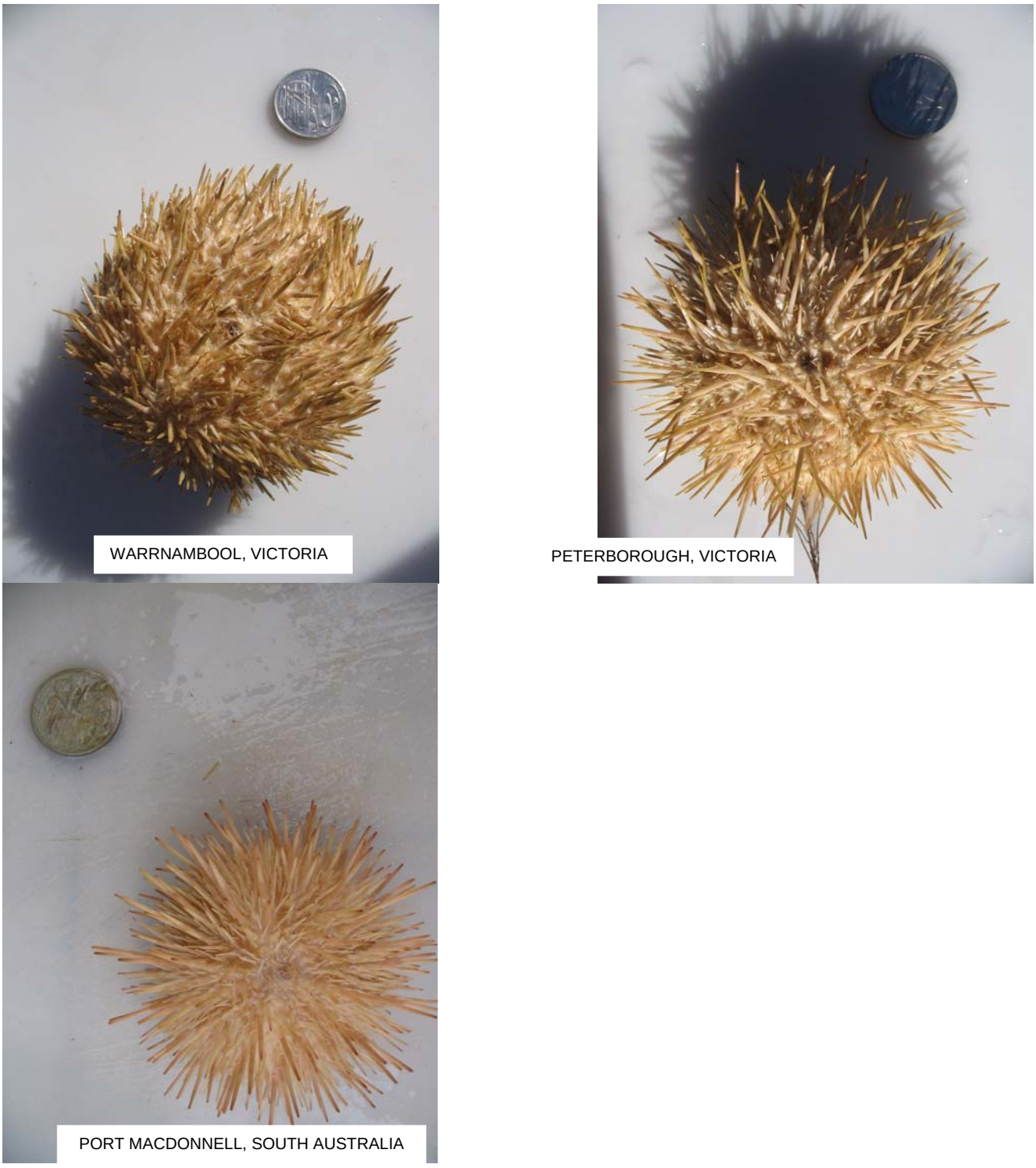

Fig. A4. Examples of white spines. It is only classified white if there is no green tinge on any part of the spine.

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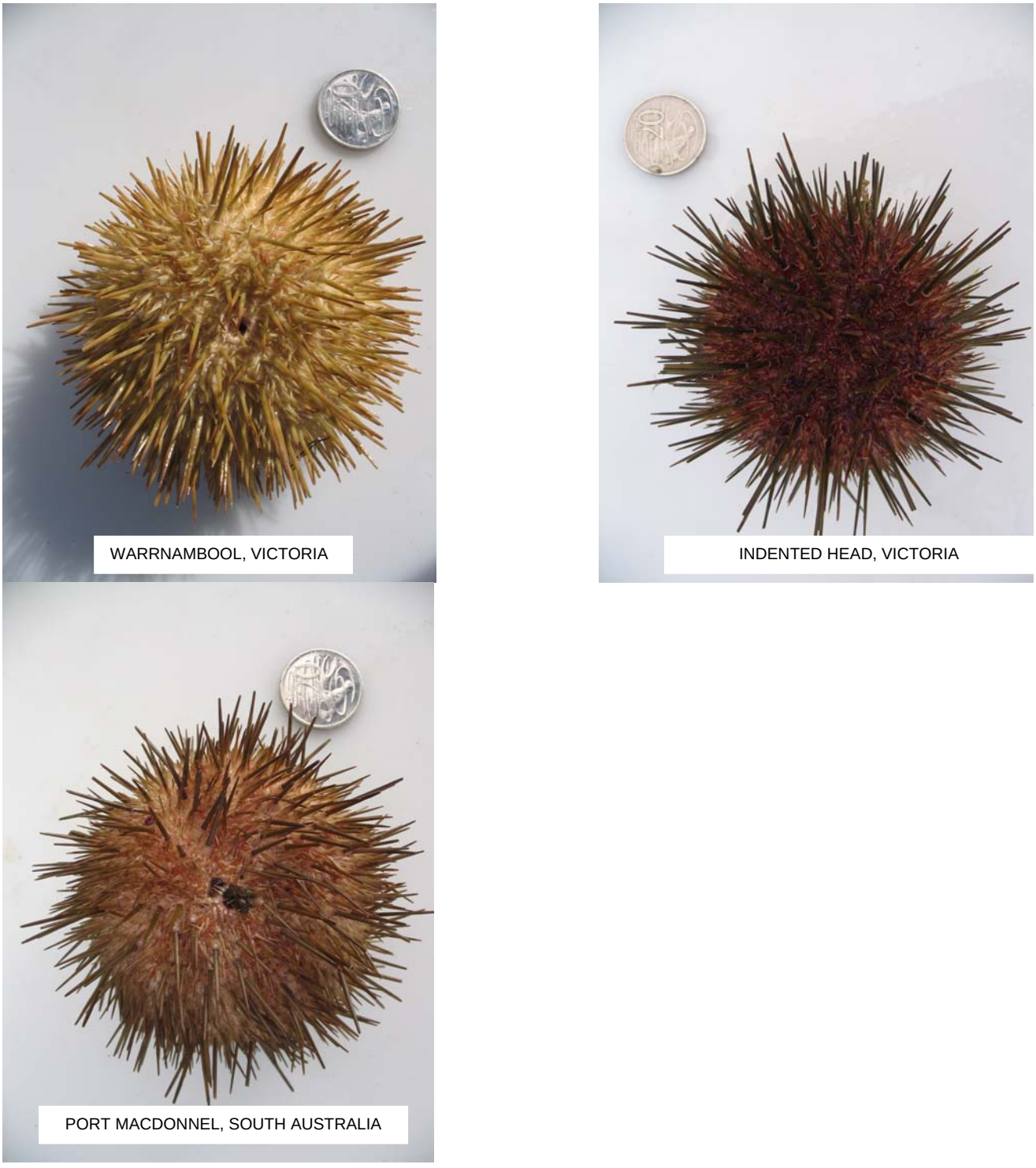

Fig. A5. Examples of green spines: green spines do not have any violet at the base.

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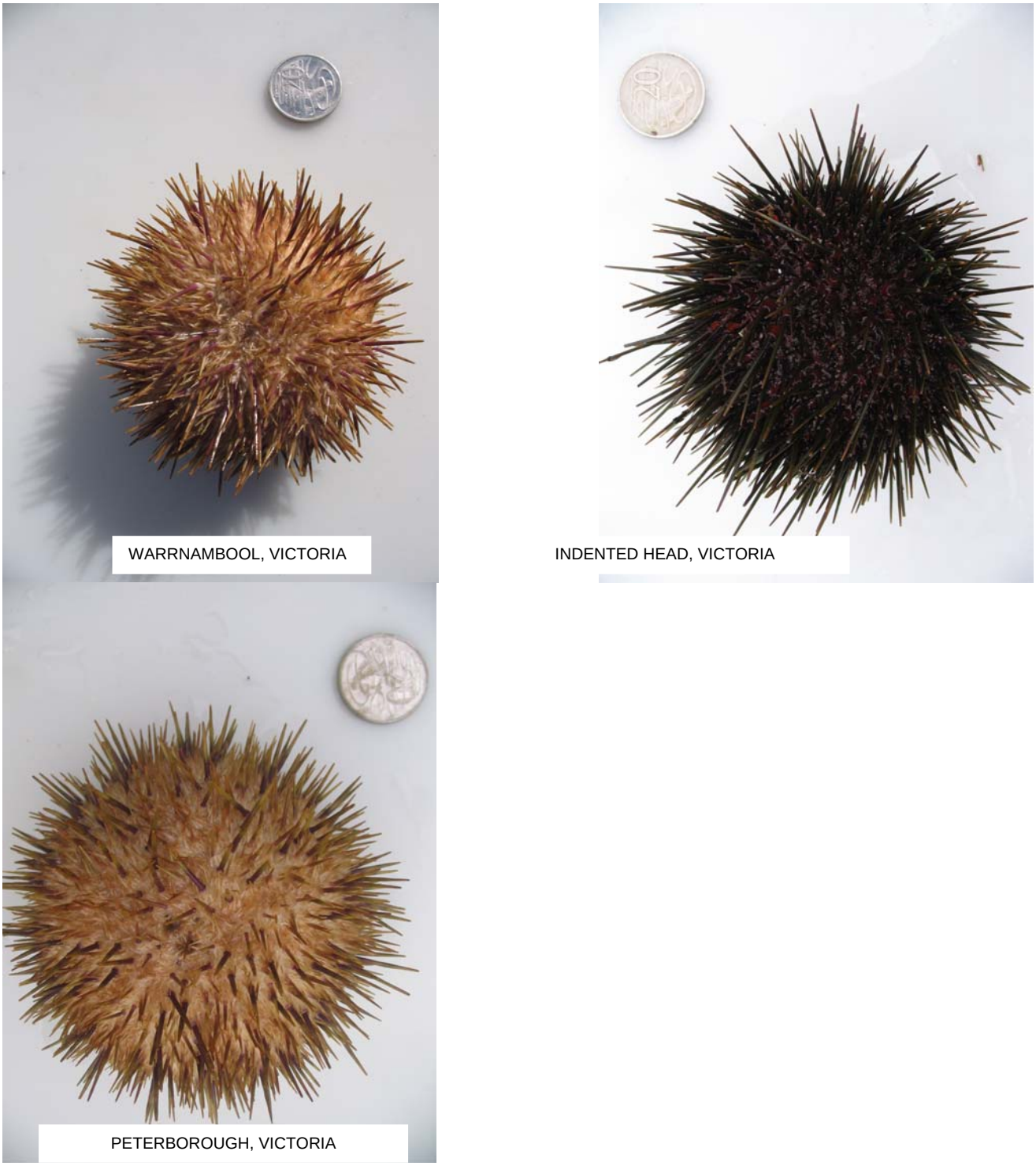

Fig. A6. Examples of violet green spines. A violet colour band is always at the base but may occur in different thicknesses. The colour then gradates into brown and/or green. Since it is difficult to identify the violet base when the test is red, it is classified violet-green if the spine gradates from a dark colour at the base to a green/brown tip (as seen here in the urchin from Indented Head, Victoria).

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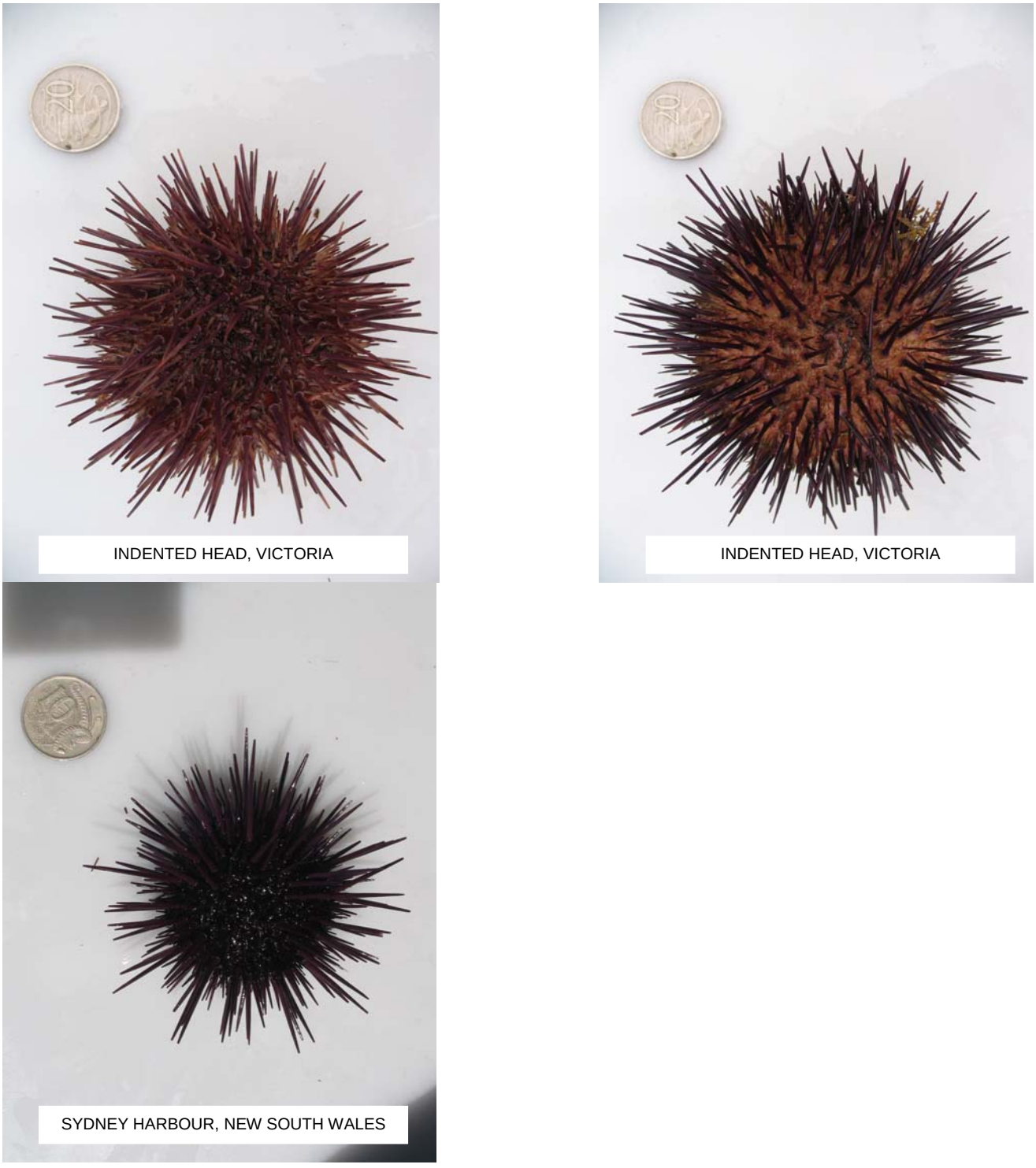

Fig. A7. Examples of violet spines. Spines were violet if this colour extended from the base of the spine to the tip.

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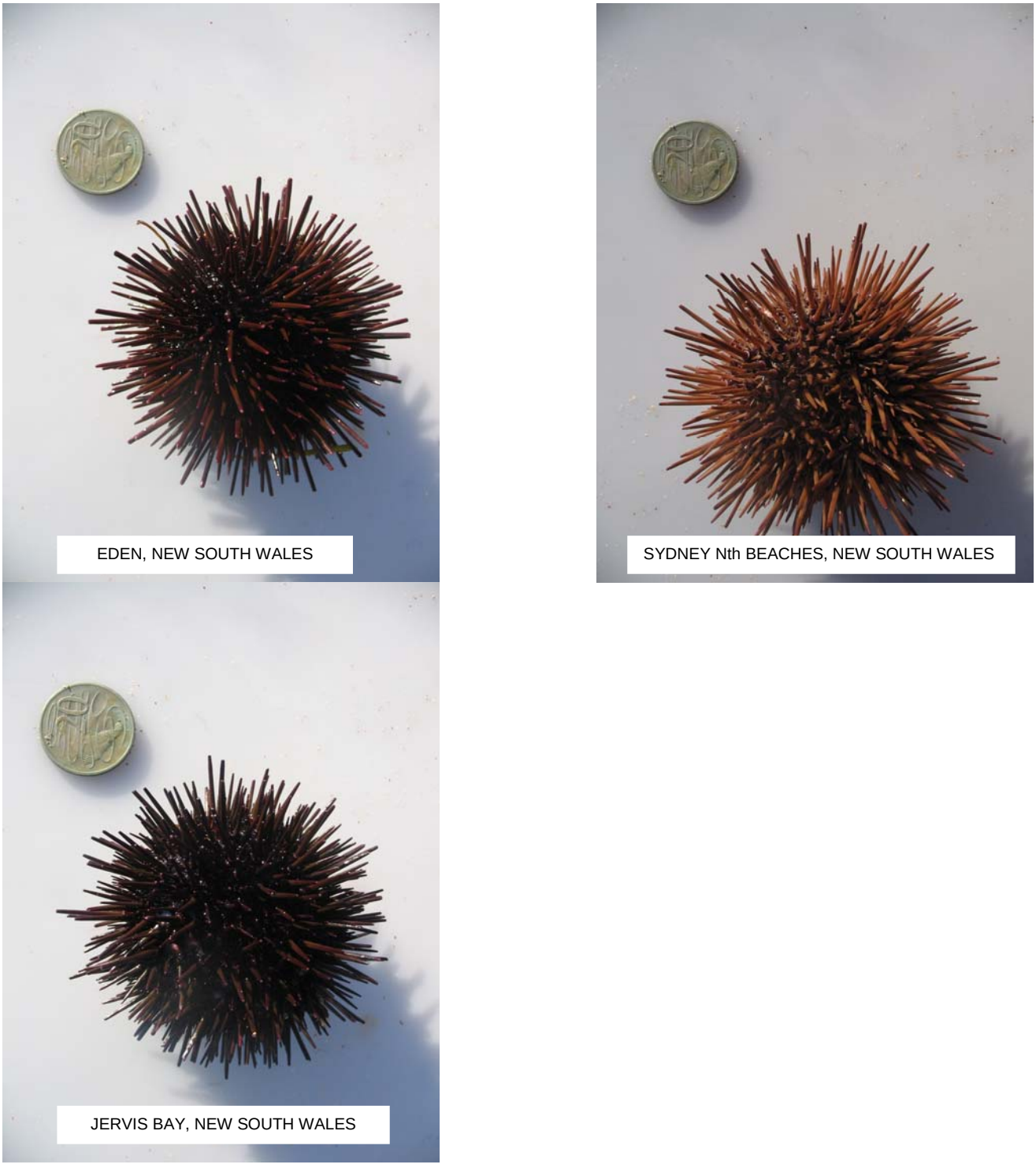

Fig. A8. Examples of violet green violet spines. Spines are classified violet green violet if the bases of the spines are violet, green in the middle of the spine and violet at the tip.

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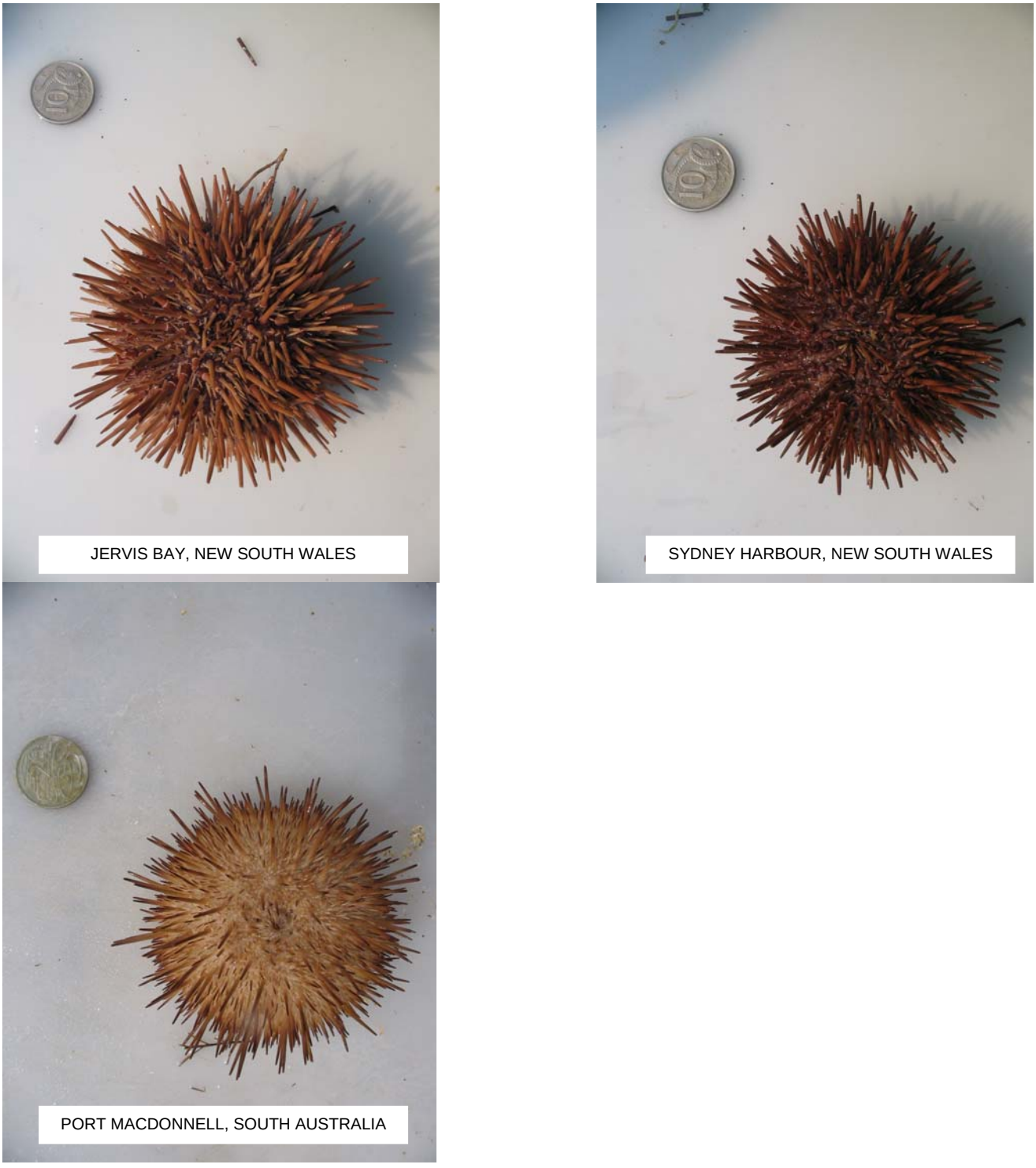

Fig. A9. Examples of brown spines. Brown spines have no green tinge and often have different shades of brown along the one spine.