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RESEARCH NOTE

# Genetic examination of the type specimen of *Ulva australis* suggests that it was introduced to Australia

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## SUMMARY

'Ana-aosa', one of the most common marine green algae in Japan, was described as *Ulva pertusa* Kjellman in 1897 from Hakodate in northern Japan. *Ulva pertusa* was considered to be a temperate species, with its native distributional range restricted to northeastern Asia. Although this species has been reported from various regions outside northeastern Asia, these records have been explained as non-indigenous populations. Recently, on the basis of genetic data and nomenclatural priority, *U. pertusa* was synonymized with *U. australis* Areschoug, a species described in 1851 from specimens collected in South Australia. Based on genetic studies, Australian populations identified as *U. pertusa* had been considered to have originated from Japan. However, the published genetic data on *U. australis* in Australia have been based only on recent collections and no historical specimens have been examined. We tested the hypothesis that native (true) *U. australis* is an independent species of very similar morphology to *U. pertusa*, but that its natural domination of shoreline habitats has been suppressed by introduced populations of *U. pertusa* from Asia. In the present study, we extracted DNA from the type specimen of *U. australis* housed in the Swedish Museum of Natural History (S) and obtained DNA sequences of the chloroplast *rbc*L gene and the nuclear rDNA ITS2 region. Our results show that *U. australis* and *U. pertusa* are genetically virtually identical, confirming that *U. pertusa* is a synonym of *U. australis.* This suggests that the introduction of *U. australis* to Australia occurred by the middle of the 19th century, when the type was collected and before there was a direct shipping route between Japan and Australia. We speculate that the introduction of *U. australis* to Australia occurred as a secondary introduction from non-indigenous populations in northeastern Asia, but not directly from Japan.

Key words: genetic examination, introduction, non-indigenous species.

*Ulva pertusa* Kjellman (Japanese name 'Ana-aosa' or 'Ana-awosa') is one of the most common intertidal green algae on temperate Japanese coasts. However, in genetic studies using recently collected specimens from various localities, *Ulva australis* Areschoug showed identical DNA sequences to *Ulva pertusa* (Kraft *et al*. 2010; Couceiro *et al*. 2011; Kirkendale *et al*. 2013). Areschoug originally described *U. australis* based on specimens collected at Port Adelaide, Australia in 1851 (Areschoug 1854), while F. R. Kjellman described *U. pertusa* based on the material collected at Hakodate (Hokkaido), 'Yenoshima' (Enoshima, Kanagawa) and Yokohama (Kanagawa), Japan (Kjellman 1897). Therefore, *U. australis* has nomenclatural priority over *U. pertusa*, and Kirkendale *et al*. (2013) synonymized *U. pertusa* with *U. australis*. Hanyuda *et al*. (2016) suggested that because *U. pertusa* is a temperate species, with its native range in


**Fig. 1.** Type specimen of *Ulva australis* Areschoug (S-A2025) housed in Swedish Museum of Natural History (S). DNA was extracted from the 5 mm × 5 mm fragments cut from the specimens in the bottom (S-A2025a) and middle right (S-A2025b) marked by black squares with white stripe. [Color figure can be viewed at wileyonlinelibrary.com]

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northeastern Asia, populations of the species outside northeastern Asia are not native. Based on genetic analyses of mitochondrial and chloroplast haplotypes, it was suggested that Australian populations originated from Japan, because Australian populations shared some of the Japanese haplotypes, which were not found from outside Japan in other parts of northeastern Asia (i.e., Korea and Russia). However, the Australian specimens of *U. australis* used for the genetic studies in Couceiro *et al*. (2011) and Kirkendale *et al*. (2013) were all relatively recently collected specimens (after 2005). Therefore, we considered the possibility that native *U. australis* is an independent species of very similar morphology to *U. pertusa*, but that its natural domination of shoreline habitats is suppressed by introduced populations of *U. pertusa* from northeastern Asia. In order to clarify the identity of *U. australis*, we have genetically examined the type specimen of *U. australis* (S-A2025) housed in the Swedish Museum of Natural History (S).

Genomic DNA was extracted from fragments of two of the four individuals on the sheet of the type specimen (S-A2025) using a QuickExtract Plant DNA Extraction Solution (Epicentre, Madison, WI, USA). We have tentatively named the type individuals as S-A2025a and S-A2025b (Figs 1–2a,b). A fragment of each specimen was ground in the extraction solution using a homogenization pestle at room temperature, followed by two-step temperature treatment according to the manufacturer's instructions. A part of the chloroplast *rbc*L gene and the nuclear rDNA ITS2 region were amplified. One primer set for *rbc*L region (rbcL-Ua1 (5′-CTTACAYTCAGGAACAGTAG-3′; annealing position corresponding to 972–991 of DQ813496 in *U. linza*) and rbcL-3.2U (5′-CCACCRAATTGTAAACATGC-3′; 1208–1189)) and two primer sets for rDNA ITS2 region (ITS2-U1 (5′-AGACCACRTCTGCCTCAGCG-3′) and ITS2-U2 (5′-GCGAGCWACCTACCTAGTCG-3′), ITS2-U3 (5′-CCGGCTGAAATRCAGAGGCT-3′) and ITS4-U (5′-CGCCGY TACTARGGGAATCC-3′)) were used for PCR amplification and DNA sequencing. PCR amplifications were carried out with a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio, Otsu, Japan) using KOD FX (ToYoBo, Osaka, Japan). After PEG purification (Lis 1980), PCR products were sequenced by a DNA sequencing service (FASMAC, Atsugi, Japan).

Due to the degraded condition of the DNA in the type specimen, only partial sequences of the *rbc*L gene (197 bp, LC331300; position 992–1188 of DQ813496 in *U. linza*) and the 5.8S rDNA + ITS2 + 28S rDNA region (242 bp, LC331301) could be determined for S-A2025b. For S-A2025a, only the 5.8S rDNA + ITS2 rDNA region (114 bp) was determined. There was no difference between the DNA sequences of the two individuals (S-A2025a and S-A2025b). Those sequences were identical to the reported sequences of *U. australis* from Australia (Kraft *et al*. 2010) and *U. pertusa* from Japan (Shimada *et al*. 2003) except for one of the *rbc*L sequences of *U. australis* (EU933957) from Queenscliff, Victoria (1 bp difference). Alignment of the sequences using the program MAFFT v.6 (Katoh & Toh 2008) is shown in Figures S1 and S2. Pairwise differences (= number of differences) were calculated using by hand or by MEGA v.6 (Tamura *et al*. 2013), and then sequence divergences (%) were manually calculated. The sequence divergences among *U. australis* (type specimen: S-A2025), *U. australis* (Australia), and *U. pertusa* (Japan) were small (Table 1; *rbc*L: 0–0.5, rDNA ITS2: 0). On the other hand, the sequence divergences between *U. australis* (type specimen) and the other phylogenetically related species (*U. lactuca* Linnaeus, *U. arasakii* Chihara, and *U. lobata* Kützing Harvey) were considerably greater (*rbc*L: 2–2.6, rDNA ITS2: 3.5–12.3).

More than 270 macroalgal species are believed to have been spread outside their native ranges by anthropogenic means (Verlaque 2001; Boudouresque & Verlaque 2002; Smith *et al*. 2002; Streftaris *et al*. 2005; Schaffelke *et al*. 2006; Williams & Smith 2007; Mineur *et al*. 2008). Among

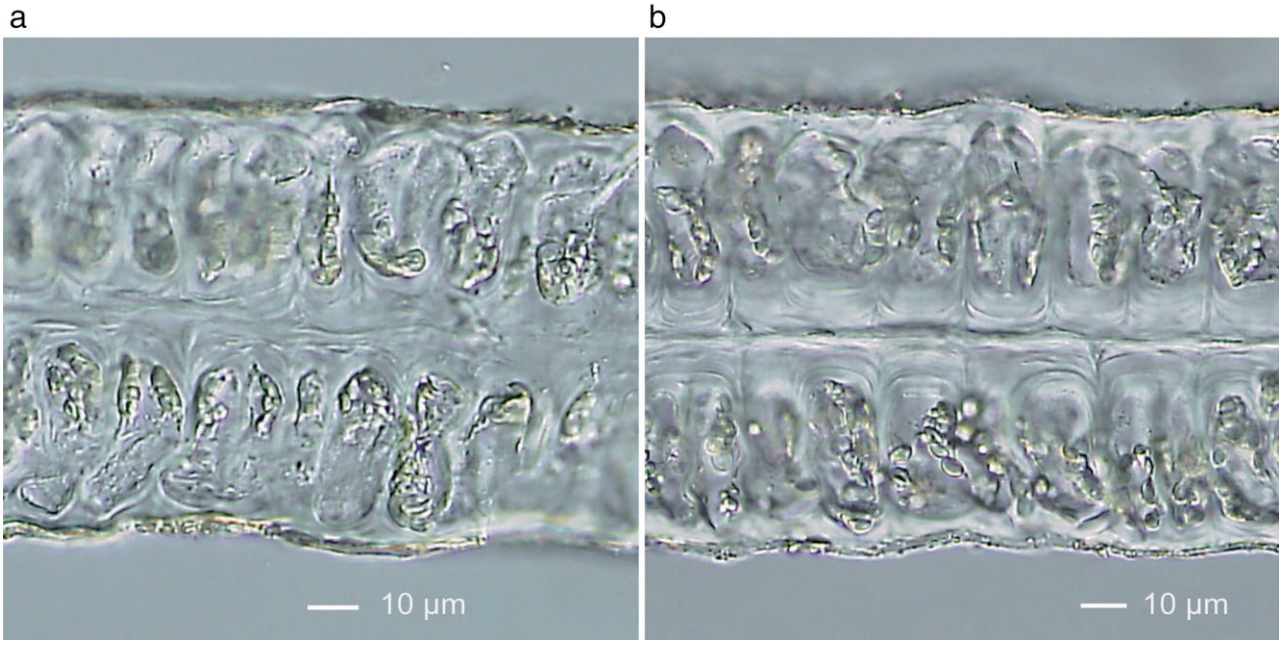


**Fig. 2.** Anatomy of type specimens of *Ulva australis* (S-A2025). (a) S-A2025a and (b) S-A2025b. [Color figure can be viewed at wileyonlinelibrary.com]

**Table 1.** Sequence divergences (%) based on the *rbc*L (above diagonal) and rDNA ITS2 (below diagonal)

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1. *Ulva australis* Areschoug (type) | — | 0–0.5 | 0 | 2 | 2.6 | 2.6 |
| 2. *Ulva australis* Areschoug (Australia)† | 0 (0)‡ | — | 0–0.5 | 2–2.5 | 2.5–3 | 2.5–3 |
| 3. *Ulva pertusa* Kjellman (Japan)§ | 0 (0)‡ | 0 (0)‡ | — | 2 | 2.5 | 2.5 |
| 4. *Ulva lactuca* Linnaerus (Japan, U.K., Canada)¶ | 6.4–6.5 (9.3–9.7)‡ | 6.4–6.5 (9.3–9.7)‡ | 6.4–6.5 (9.3–9.7)‡ | — | 0.5 | 1 |
| 5. *Ulva arasakii* Chihara (Japan)†† | 3.5 (9.8)‡ | 3.5 (9.8)‡ | 3.5 (9.8)‡ | 4.1–4.7 (11.1–11.4)‡ | — | 1 |
| 6. *Ulva lobata* Kützing Harvey (U.S.A.)‡‡ | 11.7 (12.4)‡ | 11.7 (12.4)‡ | 11.7 (12.4)‡ | 11.1–11.7 (13.5–14.4)‡ | 12.3 (18.0)‡ | — |

†rDNA ITS2 sequences: EU933980, EU933982, EU933985 (Kraft *et al*. 2010); *rbc*L sequences: EU933953, EU933954, EU933957 (Kraft *et al*. 2010).
‡The values with (inside of brackets) and without insertions/deletions.
§rDNA ITS2 sequences: AB097653, AB097654, AB097656 - AB097658 (Shimada *et al*. 2003); *rbc*L sequences: AB097624 - AB097628 (Shimada *et al*. 2003).
¶rDNA ITS2 sequences: AB097652 (Shimada *et al*. 2003), AJ234310 (Tan *et al*. 1999), AY422499 (Hayden & Waaland 2004); *rbc*L sequences: AB097623 (Shimada *et al*. 2003), AF499669 (Hayden & Waaland 2002), AY422543 (Hayden & Waaland 2004).
††rDNA ITS2 sequences: AB097650 (Shimada *et al*. 2003); *rbc*L sequences: AB097621 (Shimada *et al*. 2003).
‡‡rDNA ITS2 sequences: AY260563 (Hayden *et al*. 2003); *rbc*L sequences: AY255868 (Hayden *et al*. 2003).

them, *Ulva* species are one of the most commonly transported and widely introduced species, because of their frequent occurrence as hull-fouling species.

Low genetic diversity in *Ulva pertusa* on European and eastern Pacific coasts was noted by Wolf *et al*. (2012). Kirkendale *et al*. (2013) showed low genetic diversity among individuals/populations of *Ulva australis* from various localities in temperate Australia, and suggested the possibility that the populations in Australia were non-indigenous. Hanyuda *et al*. (2016) supported this notion by showing that the haplotypes found in Australia as well as New Zealand were dominant on the Pacific Coast of central Honshu. We suggest that the introduction of the species to Australia from northeastern Asia (plausibly originated from central Japan judging from the haplotypes) dates back to the early 19th century. However, Japan was a closed country for about two centuries until the middle 19th century, and there was no direct shipping between Japan and Australia during this period. The only international shipping during the period was between Nagasaki, Kyushu and China and some southeastern Asian countries, or between Tsushima and Korea. In contrast, there was relatively frequent shipping among Europe, Australia and China. However, the first introduction of '*Ulva pertusa*' to Europe was estimated to be the early 1970s (Verlaque *et al*. 2002). Similarly, based on the genetic analyses of old specimens collected from the Mediterranean, Wolf *et al*. (2012) concluded that the introduction of '*Ulva pertusa*' to the area was later than 1932. Therefore, it is not likely that the species was directly transported from Europe to Australia in the 19th century. Consequently, we speculate that the introductions of *U. australis* to Australia (and perhaps also to New Zealand) occurred as secondary introduction from non-indigenous populations in northeastern Asia, but not directly from Japan.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** Sequence alignment of partial *rbc*L sequences of the type specimen of *Ulva australis* and related taxa.

**Fig. S2.** Sequence alignment of 5.8S rDNA + ITS2 + 28S rDNA region sequences of the type specimen of *Ulva australis* and related taxa.