EDINBURGH JOURNAL OF BOTANY 79, Article 1925: 1–15 (2022). https://doi.org/10.24823/EJB.2022.1925

Published by the Royal Botanic Garden Edinburgh
ISSN (online): 1474-0036, ISSN (print): 0960-4286

# YELLOW WATER LILIES (*NUPHAR*, NYMPHAEACEAE) IN GREAT BRITAIN: A NEW HYBRID, A REAPPRAISAL OF RECORDS, AND A REVISED STATUS OF *N. ADVENA*

R. V. Lansdown [1,2] & M. Ruhsam [3]

Surveys to clarify the identification of the alien spatterdock (*Nuphar advena*) in Great Britain (England, Scotland and Wales) showed that most reported populations included plants that did not have a morphology that agreed with literature accounts of that species. Some populations had been misidentified, whereas others were morphologically atypical variants of the UK native *Nuphar lutea*. Many populations included individuals that had a combination of traits characteristic of both *Nuphar advena* and *N. lutea*. Visual examination and measurement of two stamen characters (filament and anther length) show that these plants are sterile, and molecular analysis confirms that these populations include a previously undescribed sterile hybrid between *Nuphar advena* and *N. lutea*. Here, we formally describe the hybrid as *Nuphar* × *porphyranthera* and provide taxonomic and distribution information on the parental species for comparison, as well as a key to all known *Nuphar* taxa in Great Britain. Neither *Nuphar advena* nor *Nuphar* × *porphyranthera* show any sign of spreading from sites at which they were almost certainly planted and therefore should not be considered naturalised in Britain.

*Keywords*. Hybridisation, non-native, *Nuphar* × *porphyranthera*, *Nuphar lutea*, ornamental aquatic plant.
Received 2 February 2022 Accepted 3 November 2022 Published 15 December 2022

## Introduction

The genus *Nuphar* Sm. (Nymphaeaceae) includes 11 species, 3 subspecies and 4 hybrids and is native to fresh waters of the northern hemisphere (Padgett, 2007). In Great Britain two native species, namely *Nuphar lutea* (L.) Sm. and *N. pumila* (Timm) DC. (and their natural hybrid *Nuphar* × *spenneriana* Gaudin), occur, as well as *N. advena* (Aiton) W.T.Aiton var. *advena*, a species introduced from eastern North America (Stace, 2019).

As part of ongoing studies to clarify the status and identification of aquatic plants in Great Britain, fieldwork carried out by the first author visiting sites with records of *Nuphar advena* in the Botanical Society of Britain & Ireland (BSBI) database (https://database.bsbi.org/maps/?taxonid=2cd4p9h.gms) raised questions about the accuracy of some of the records, because anther and filament measurements were inconsistent with those given in the literature. The BSBI database includes 26 separate populations in the UK where *Nuphar*

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*advena* has been recorded (Table 1, Figure 1). Most of these populations are in ornamental lakes, but there are also a few in natural or seminatural water bodies occurring between Hampshire in England and East Inverness-shire in Scotland.

At all the populations visited, the distribution of plants, as well as anther and filament measurements, suggested that only a single *Nuphar* species was present. It soon became clear that some populations recorded as *Nuphar advena* had been misidentified, however; many populations included a taxon that was neither the introduced *N. advena* nor the British native *N. lutea* but had a combination of traits characteristic of both, suggesting a hitherto undescribed hybrid between the two. Here, we present a revision of the current status of

**Table 1.** Unique records of *Nuphar advena* populations in the Botanical Society of Britain & Ireland database

| Sample | Field ID | Genetic ID | Location | VC | NGR |
|---|---|---|---|---|---|
| NA1 | *Nuphar advena*[a] | *Nuphar advena* | Spottiswoode Loch | 81 | NT610493 |
| NL1 | *Nuphar lutea* | *Nuphar lutea* | Swanwick NR | 11 | SU508100 |
| Pop1 | *Nuphar lutea* | NA | Ashtead Park, S pond | 17 | TQ191583 |
| Pop2 | *Nuphar lutea* | NA | Ashtead Park, N Pond | 17 | TQ191585 |
| Pop3 | *Nuphar lutea* | NA | Aymestry Pond | 37 | SO814541 |
| Pop4 | *Nuphar lutea* | NA | Brook House, Clun | 40 | SO271793 |
| Pop5 | *Nuphar lutea*[a] | NA | Carlingwark Loch | 73 | NX7661 |
| NL3 | *Nuphar lutea*[a] | *Nuphar lutea* | High Gooseloan | 75 | NS3246 |
| NH1 | Putative hybrid[a] | Hybrid | West Hoathly Area | 14 | TQ364339 |
| NH2 | Putative hybrid[a] | Hybrid | Godstone, Glebe Water | 17 | TQ358514 |
| NH3 | Putative hybrid[a] | Hybrid | Windsor Great Park, Cow Pond | 17 | SU975714 |
| NH4 | Putative hybrid[a] | Hybrid | Painshill Park, The Lake | 17 | TQ095599 |
| NH5 | Putative hybrid[a] | Hybrid | Shepperton, Ferry Lane pit | 21 | TQ076663 |
| NH6 | Putative hybrid[a] | Hybrid | Shropham Ponds | 28 | TL971933 |
| NH7 | Putative hybrid | Hybrid | Sketty Hall, Swansea | 41 | SS623943 |
| NH8 | Putative hybrid | Hybrid | Oulton Park, main pond | 63 | SE36122768 |
| NH9 | Putative hybrid[a] | Hybrid | Corehouse Estate | 77 | NS87764210 |
| NH10 | putative hybrid[a] | Hybrid | Larriston Lime Works | 80 | NY557938 |
| NH11 | Putative hybrid[a] | Hybrid | Black Loch | 96 | NH98645551 |
| NH12 | Putative hybrid[a] | Hybrid | Kirkhill, Auchnagairn House | 96 | NH55754486 |
| Pop6 | *Nymphoides peltata* | NA | Hitterhill Coppice, Wyre Forest | 37 | SO767761 |
| Pop7 | *Nymphoides peltata* | NA | Snuffmill Pond | 37 | SO784746 |
| Pop8 | Population lost | NA | Virginia Water, Village Pond | 17 | SU991698 |
| Pop9 | Population lost | NA | Godstone, Horse Pond | 17 | TQ350515 |
| Pop10 | Unconfirmed (inaccessible) | NA | Chilworth, Tangley Mere | 17 | TQ027471 |
| Pop11 | Unconfirmed (not visited) | NA | Loch Ard | 87 | NN491015 |

Field ID, revised identification after field visits by the first author (R.V.L.); Genetic ID, genetic identification based on nrITS2 sequences; NA, not applicable; NGR, National Grid reference; VC, vice-county number.
[a] Populations from which biometric data were collected: 10 samples (*n*) per population apart from NH2, with *n* = 20. Additional biometrics were collected from a population of *Nuphar lutea* at Ferry Lane, Chertsey (*n* = 10, TQ041677).

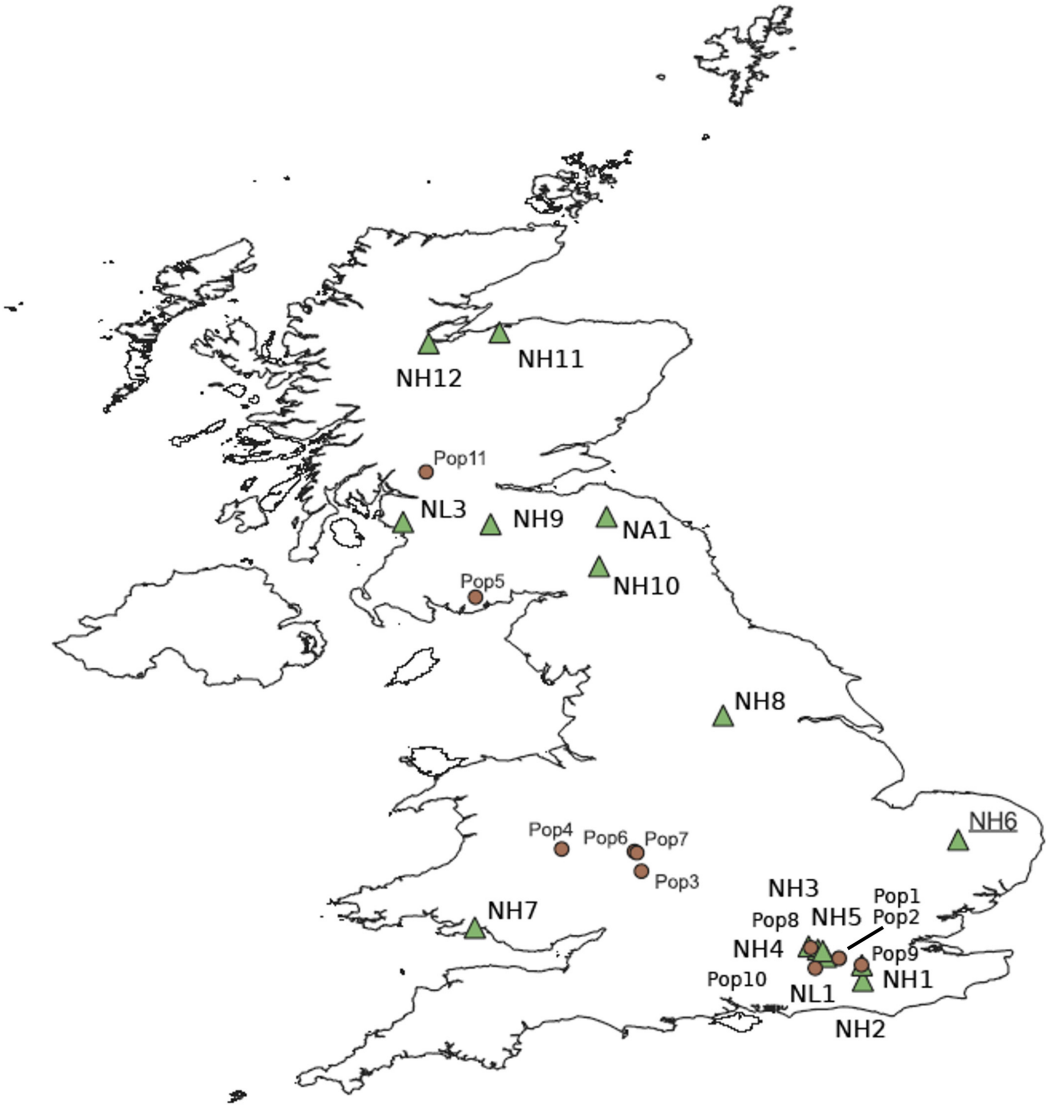


**Figure 1.** Location of the 26 *Nuphar advena* records in the Botanical Society of Britain & Ireland database. Populations sampled for genetic analysis are marked with a triangle.

*Nuphar advena* in the UK, describe a new hybrid in the genus *Nuphar*, and provide a key for the identification of all *Nuphar* taxa known to occur in Great Britain and Ireland.

## Materials and methods

Of the 26 populations in Great Britain where *Nuphar advena* has been recorded, 24 were visited, covering 90% of its known range, to ascertain the identity of plants that did not

satisfactorily fit the morphology of *N. advena* nor any other British *Nuphar* taxon. To do this, we used a genetic and morphological approach.

### *Genetics*

Following the manufacturer's protocol, the DNeasy Plant kit (Qiagen, Hilden, Germany) was used to extract DNA from collected leaf samples stored in silica gel. The nuclear DNA-barcoding marker internal transcribed spacer 2 (ITS2, Hollingsworth *et al.*, 2011) was used to distinguish between *Nuphar lutea* and *N. advena*. Downloaded sequences of the available ITS2 sequences for both species from the National Center for Biotechnology Information (GenBank, https://www.ncbi.nlm.nih.gov/nuccore/) showed that the two species differed by nine single nucleotide polymorphisms (SNPs) along a c.300 bp stretch of ITS2. The downloaded sequences included four *Nuphar advena* (AH006158, FM242145, MG234973, MG236858) and 13 *N. lutea* samples (AH006165, AY620427, EU428043, FM242147, KX165445, KY972340, KY972343 and MT300330–MT300335). ITS2 sequence data were obtained for all samples identified as putative hybrids between *Nuphar lutea* and *N. advena* in the field ($n$ = 12) based on growth form and flower characters, as well as one individual identified as *N. advena* and two individuals identified as *N. lutea* (see Table 1).

Polymerase chain reactions (PCRs) of ITS2 were carried out using the primers S2F and S3R, as detailed in table S1 in Chen *et al.* (2010). Purified PCR products were sequenced at DNA Sequencing and Services (University of Dundee), using a Big Dye sequencing mix (Applied Biosystems, Waltham, MA, USA) and the PCR primers. Complementary DNA sequence strands were then assembled and aligned using the software Geneious Prime2019 (Biomatters, Auckland, New Zealand).

### *Morphology*

Of the 24 populations visited, flowering plants were available and accessible from 13 (see **Table 1**; plant records found to be misidentifications were excluded). In these populations, measurements were taken from 10 plants per population (apart from NH2, having 20 plants). These measurements included sepal number, the lengths of 10 filaments, and the lengths of 10 anthers from one flower per plant (**Figure 2**). Measurements were taken from one individual plant for each trait and the mean was calculated. These characters were selected because they appeared to provide the most reliable distinction between *Nuphar advena* and *N. lutea*, based on the literature (e.g. Padgett, 2007). Additionally, the presence of fertile (dehiscent) or infertile (aborted, with no pollen formed) anthers was established on as many flowers as could be readily accessed ($n$ = 10). Qualitative traits such as petiole shape, habit of leaves (emergent or floating), anther and pollen colour, as well as capsule shape, were also noted.


**Figure 2.** A, Flower of *Nuphar* × *porphyranthera* with some stamens removed, from NH8 population, Oulton Park, June 2021. B, Sterile stamens of *Nuphar* × *porphyranthera*: filament length (fl), anther length (al). C, Fertile stamens of *Nuphar advena*. Scale bars are approximate (no measurements of the photographed features were taken). Photographs: R. V. Lansdown.

## Results

Of the 15 genotyped samples (see Table 1) from populations that were originally recorded as *Nuphar advena* in the BSBI database, only one sample (NA1) matched the expected genotype of that species. Twelve samples (NH1–NH12) that were identified during field visits as possible hybrids between *Nuphar advena* and *N. lutea* clearly showed the genetic signature of a hybrid. All nine species-specific SNPs of the parental species were heterozygous in the hybrids, that is, they carried the species-specific SNP of each parental species, visible as double peaks in the electropherograms. Two samples (NL1 and NL3) that were identified as *Nuphar lutea* in the field matched the genetic signature of that species.

Anther and filament lengths showed substantial variation between and within taxa, however; the *Nuphar advena* population of NA1 had consistently short filaments compared with the other two taxa (Table 2, Figure 3). Putative hybrid samples covered the whole spectrum of the parental species, but filament length (mean, 8.1 mm; range, 5–11 mm) was not as low as the shortest *Nuphar advena* filament (4.2 mm) nor as high as the longest *N. lutea* filament (12 mm). By contrast, the maximum anther length of the hybrid exceeded the length of both parents (see Table 2, Figure 3); all anthers in genetically confirmed hybrid individuals were sterile (indehiscent), with no pollen developing.

## Discussion

Our results suggest that of the 26 recorded *Nuphar advena* populations in the UK, only one (Spottiswoode Loch, Berwickshire) is actually *N. advena*. Eight populations were misidentified and were *Nuphar lutea*, and two populations were actually the fringed water lily, *Nymphoides peltata* (S.G.Gmel.) Kuntze. The population at Loch Ard was not visited in 2020–2021 due to lack of time, one population could not be assessed because it was within the grounds of a private house, and two populations had been lost due to re-excavation or bank works on the

**Table 2.** Morphological characters of British populations of *Nuphar advena*, *Nuphar* × *porphyranthera* and *N. lutea*

| Character | *N. advena* | *Nuphar* × *porphyranthera* | *N. lutea* |
|---|---|---|---|
| No. of samples (from *n* populations) | 10 (1) | 110 (10) | 30 (3) |
| Petiole shape | Rounded-angular | Rounded or angular | Triangular |
| Leaves | Emergent | Emergent and floating | Emergent and floating |
| No. of sepals | 6 | 5 or 6 | 5 |
| Anther length (mm)[a] | 7.0 (6.3–7.8) | 6.4 (4.1–9.9) | 6.8 (5.4–9.0) |
| Filament length (mm)[a] | 5.1 (4.2–5.8) | 8.1 (5–11) | 8.1 (4.8–12) |
| Ratio of anther to filament length[a] | 1.4 (1.3–1.6) | 0.8 (0.5–1.3) | 0.9 (0.6–1.5) |
| Anther colour | Dark purple | Purple | Yellow |
| Pollen colour | Yellowish white | NA | Yellow |
| Capsule shape | Ovoid-obovate | NA | Urceolate |

NA, not applicable.
[a] Mean (range).

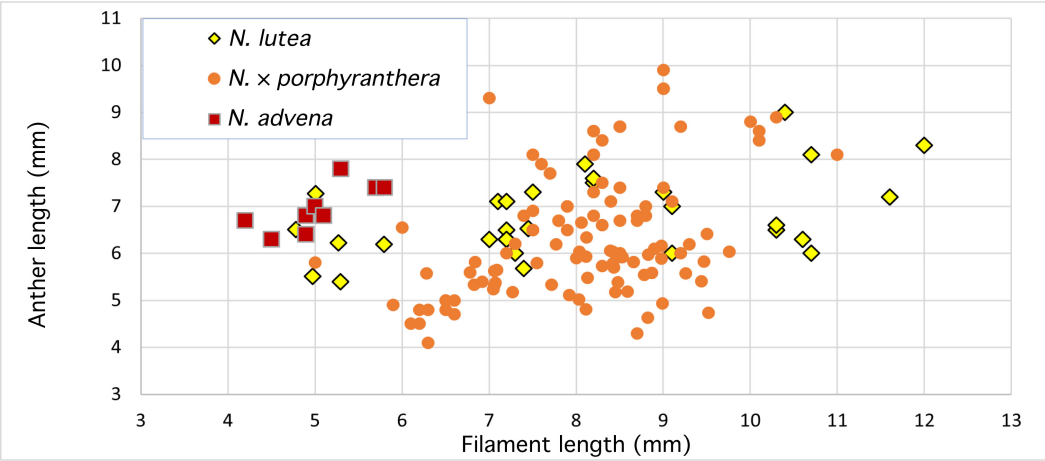


**Figure 3.** Anther and filament lengths of *Nuphar lutea* (*n* = 30 from 3 populations), *N. advena* (*n* = 10 from 1 population) and their hybrid *Nuphar* × *porphyranthera* (*n* = 100 from 10 populations).

ponds. Nevertheless, our findings indicate that the introduced species *Nuphar advena* is much rarer in Great Britain than BSBI records have suggested.

The other 12 populations were genetically confirmed to consist of a hitherto undescribed hybrid between *Nuphar advena* and *N. lutea* and included plants with mainly emergent leaves and purple, sterile anthers. All confirmed populations of *Nuphar advena* and the genetically identified hybrid between *N. advena* and *N. lutea* occurred in ornamental ponds, apart from the Shepperton population (NH5); this was in a gravel pit where it was almost certainly planted, because the closest populations are more than 40 km apart and there is no

evidence that either of these taxa has spread from populations to which they were probably introduced. An unconfirmed population in a natural pond on Stoke Common in Berkshire was probably of hybrid origin but died out before it could be sampled for this study.

Our results highlight that morphological variation in the Great British native *Nuphar lutea* is greater than generally recognised. Not only are there populations with a reasonably large proportion of leaves emergent (e.g. at Carlingwark Loch), but the relative and absolute dimensions of reproductive parts show greater variation than described in the published literature (Padgett, 2007).

Here, we describe and name the hitherto unknown hybrid between *Nuphar advena* and *N. lutea* and provide an updated account of the parental species as well as a key to all *Nuphar* taxa known from Great Britain. For a detailed account of *Nuphar pumila* and *Nuphar* × *spenneriana* in Great Britain, see Lansdown (2017).

## Taxonomic treatment

**Nuphar × porphyranthera** Lansdown and Ruhsam, **hybr. nov.**
(*N. lutea* × *N. advena*)

The most useful characters for distinguishing *Nuphar* × *porphyranthera* from *N. advena* and *N. lutea* are anther sterility and the failure to develop capsules, the number of sepals, petiole shape in section, and the proportion of leaves that are emergent or floating. The early degradation of leaves in July, possibly caused by an invertebrate to which the hybrid may be more susceptible, is also a good indicator of populations of *Nuphar* × *porphyranthera* (Figure 4), because *N. lutea* and *N. advena* leaves typically persist at least until September. *Nuphar* × *porphyranthera* populations that occur in lakes which are no longer maintained and drying out, typically have swards of upright leaves (Figure 5C). Based on a limited amount of available material, it appears likely that *Nuphar* × *porphyranthera* can be distinguished from *N. lutea*, even when not flowering, by the shape of the petiole section (Figure 6B). Holotype: United Kingdom. Middlesex. Small stand in shade of trees on margin of former gravel pit, Ferry Lane, Shepperton, TQ076663, 7 vii 2021, *R.V. Lansdown* RVL 21/1, E01080171 (E). Paratype: United Kingdom. Surrey. Glebe Water, Godstone, TQ3584851407, 16 vii 2022, *R.V. Lansdown* RVL 21/2 (BM). **Figures 4, 5, 6B, 7B, 8B.**

Most *leaves* emergent, vertical or at a steep angle (Figure 5), leaves that lie flat on the water often forming a fringe around stands of emergent leaves, starting to decay in July with blade generally lost by August; *petiole* rounded or angular in section (Figure 6B). *Sepals* 5 (or 6), the 2 or 3 outer mainly green, the 3 inner yellow with a green patch at the base, occasionally tinged reddish on the margins (Figure 7B). *Filaments* 4.1–9.9 mm, typically yellow to whitish yellow; *anthers* 5–11 mm, typically deep purple, occasionally yellow (Figure 8B), with two raised lines but no pollen formed; ratio of anther to filament length, 0.5–1.3. *Capsules* aborted (i.e. misshaped and shrunken).

**Figure 4.** Habit of *Nuphar* × *porphyranthera*, showing decaying leaves in July 2020, from NH4 population, Painshill Park. Photograph: R. V. Lansdown.

***Nuphar advena*** (Aiton) W.T.Aiton

Most *leaves* emergent, vertical or at a steep angle, few (if any) lying flat on the water (see Figure 9), typically persistent at least until September; *petiole* rounded or angular in section (Figure 6A). *Sepals* 6, the 3 (or 4) outer mainly green, the 3–8 inner yellow with a green patch at the base, occasionally tinged reddish to peach coloured (Figure 7A). *Filaments* 6.3–7.8 mm, pale yellow; anthers 4.2–5.8 mm, deep purple with two lines of whitish yellow pollen (Figure 8A); ratio of anther to filament length, 1.3–1.6. *Capsules* developed, ovoid to broadly obovate, which contrasts with the urceolate shape in *Nuphar lutea*.

*Material examined*. United Kingdom. Berwickshire. Aquatic herb with leaves erect, standing proud of water surface; *outer tepals* externally green at base and red or yellow above, red within; *inner tepals* yellow, suffused reddish within; *stigmatic column* yellow on top. Lake margin; growing in water, Spottiswoode Loch (Berwickshire, UK) east side, NT61034928, 19 vii 2021, *D.G. Long* No. 47192 with R.V. Lansdown, E01004414 (E).

*Nuphar advena* is currently known from a single confirmed site in Britain in the lake on the Spottiswoode Estate, Berwickshire, where it was first recorded in 1964 (Long, 1998, 2020). It forms two large stands along the margin of one side of the lake.


**Figure 5.** *Nuphar* × *porphyranthera*: A, leaf shape from NH12 population, Auchnagairn House; B, leaf shape from NH6 population, Shropham Ponds; C, habit from NH12 population in drying-out pond, Auchnagairn House. Photographs: R. V. Lansdown.

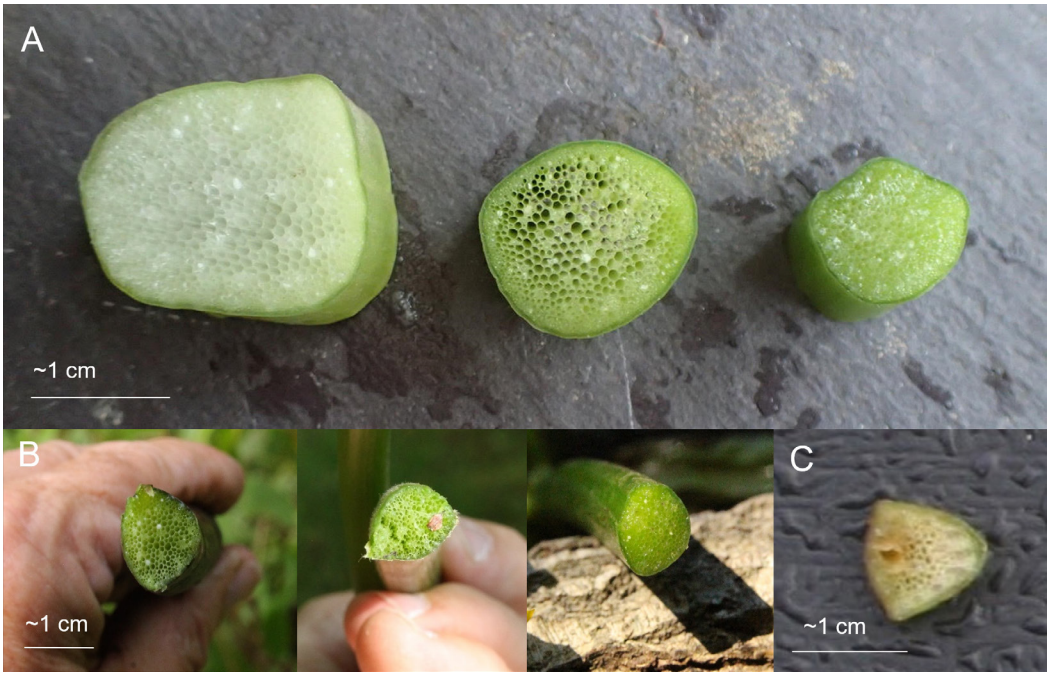


**Figure 6.** A, Petiole shape of *Nuphar advena* from NA1 population, Spottiswoode Loch. B, Petiole shape of *Nuphar* × *porphyranthera* from NH8 population, Oulten Park (left); NH6 population, Shropham Ponds (centre); and NH1 population, West Hoathly (right). C, Petiole shape of *Nuphar lutea* (no population code), Elterwater. Scale bars are approximate (no measurements of the photographed features were taken). Photographs: R. V. Lansdown.

***Nuphar lutea*** (L.) Sm

Most leaves submerged or floating, occasionally emergent but these scattered or patchy within stands (Figure 10), typically persistent at least until September; *petiole* triangular in section (Figure 6C). *Sepals* 5, the 2 or 3 outer mainly green, the 3 inner yellow with a green patch at the base (Figure 7C). *Filaments* 5.4–9 mm, whitish to pale yellow; *anthers* 4.8–12 mm, yellow with two lines of yellow pollen (Figure 8C); ratio of anther to filament length, 0.55–1.5. *Capsules* very frequent, urceolate.

*Nuphar lutea* is widespread and locally abundant throughout Scotland north of the Great Glen but sparse at higher altitudes (Preston *et al.*, 2002).

## *Key to Nuphar species in Great Britain*

1a. Sepals 6 (usually 3 or occasionally 4 green outer and 3–8 yellow inner) _____ 2
1b. Sepals 5 (usually 2 green outer and 3 mainly yellow inner) _____ 3

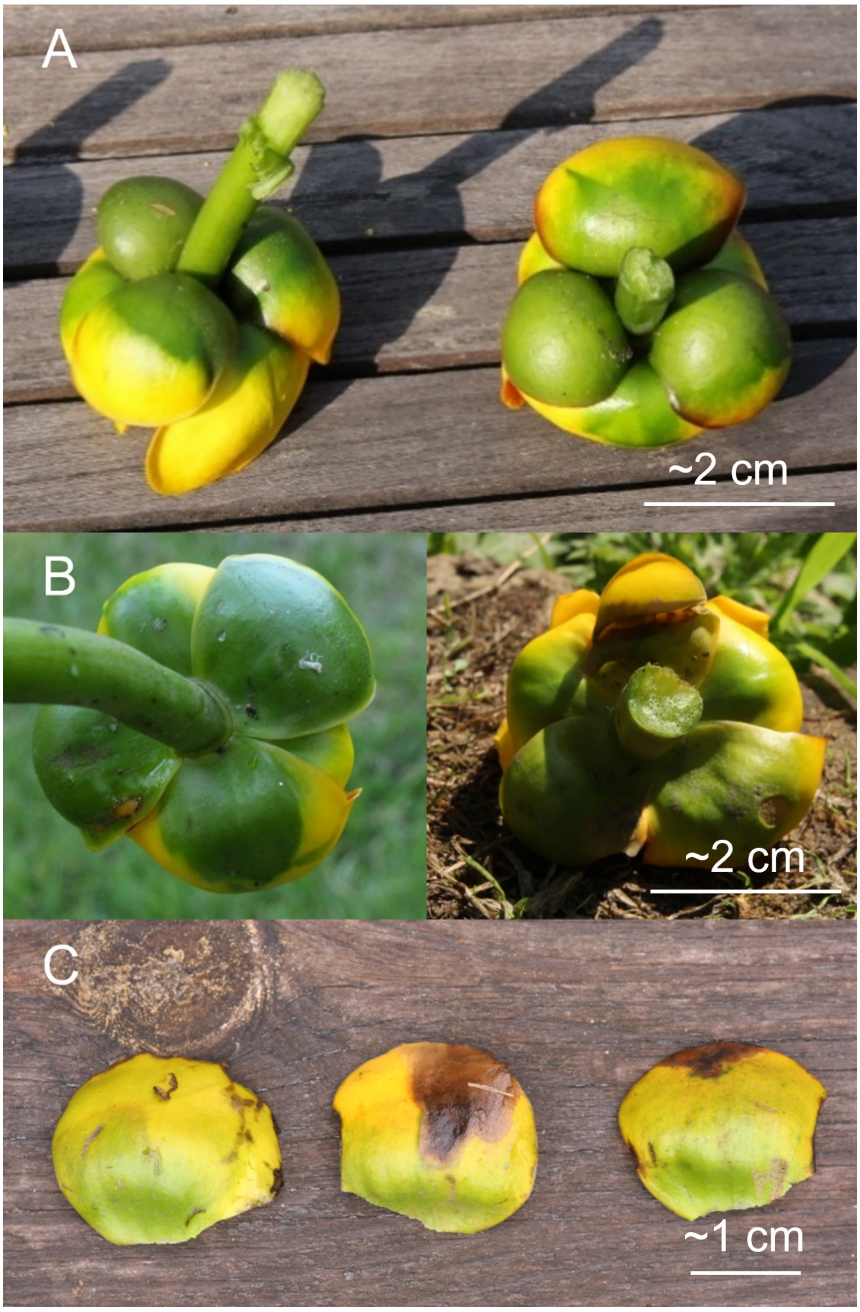


**Figure 7.** Sepals of: A, *Nuphar advena* from NA1 population, Spottiswoode Loch; B, *Nuphar* × *porphyranthera* from NH8 population, Oulten Park (left), and NH2 population, Godstone (right); C, *N. lutea* (no population code), Norbotten, Sweden. Scale bars are approximate (no measurements of the photographed features were taken). Photographs: R. V. Lansdown.

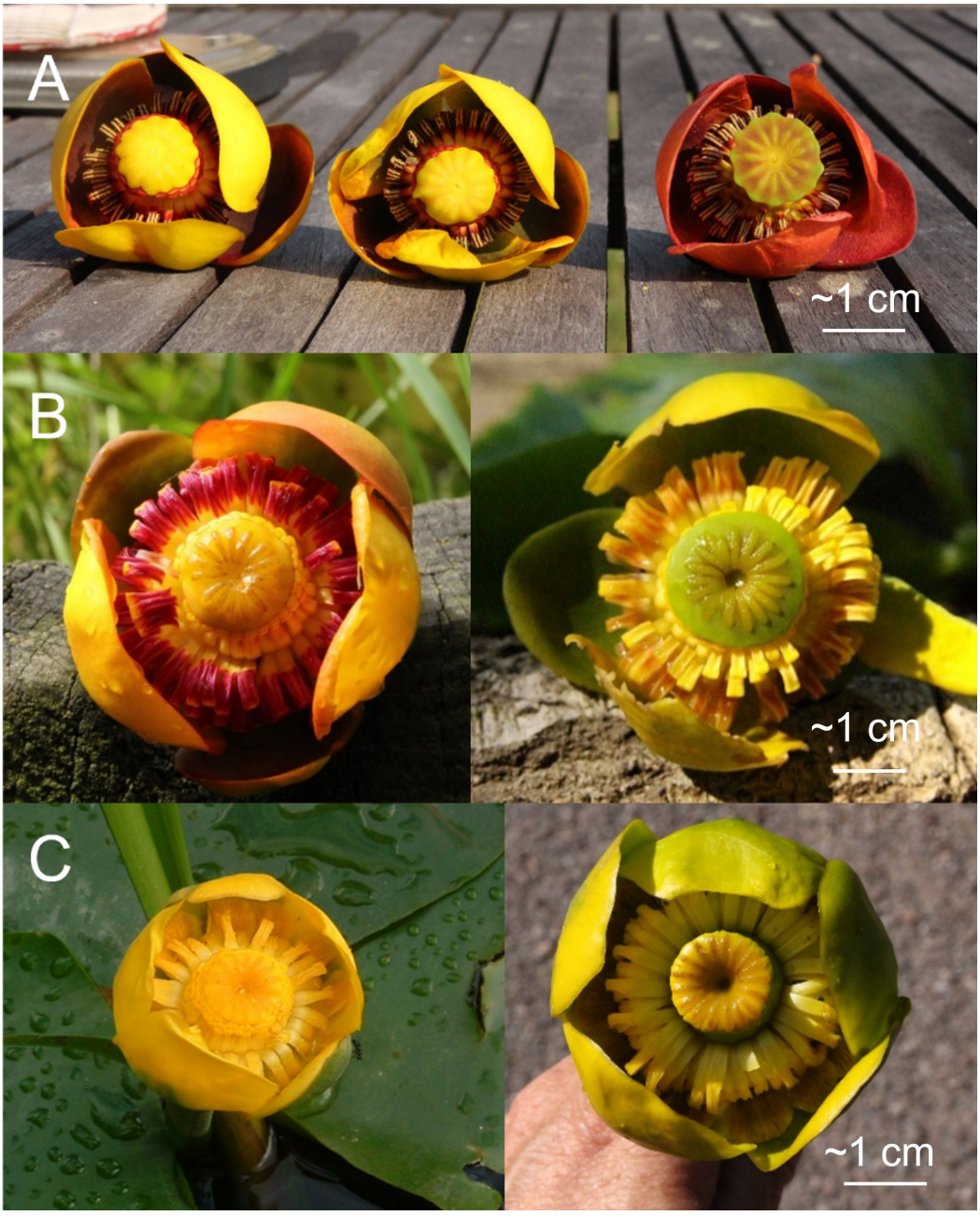


**Figure 8.** Flowers and stamens of: A, *Nuphar advena* from NA1 population, Spottiswoode Loch; B, *Nuphar* × *porphyranthera* from NH8 population, Oulten Park (left), and NH1 population, West Hoathly (right); C, *N. lutea* (no population code), Elterwater (left) and Pop1 population, Ashtead (right). Scale bars are approximate (no measurements of the photographed features were taken). Photographs: R. V. Lansdown.

**Figure 9.** Habit of *Nuphar advena*, showing leaves in July 2021, from NA1 population, Spottiswoode Loch. Photograph: R. V. Lansdown.

2a. Anthers not developing, of uniform colour, reduced to two raised lines at the end of the filament; fruit not produced; leaves typically degrading in July ___ *Nuphar* × *porphyranthera*

2b. Anthers dehiscing, pollen yellowish white in two lines, contrasting with the colour of the anther; fruit produced; leaves persisting at least into September _____ *N. advena*

3a. Petiole rounded to angular in section; most leaves emergent, floating leaves often forming a fringe around stands dominated by emergent leaves, typically degrading in July; capsules not forming but decaying before development _____ *Nuphar* × *porphyranthera*

3b. Petiole triangular in section; most or all leaves floating or submerged, floating leaves throughout stands, persisting at least into September; capsule typically developing, urceolate _____ 4

4a. Leaves with 23–28 lateral veins on each side; stigmatic disc 10–15 mm in diameter, typically circular or slightly crenate at margins, with 9–24 rays; flowers 3–6 cm in diameter _____ *N. lutea*

4b. Leaves with 11–22 lateral veins on each side; stigmatic disc 6–11 mm in diameter, crenate to lobed at margin, with 7–14 rays; flowers 1.5–4 cm in diameter _____ 5

5a. Leaves with 15–22 lateral veins on each side; stigmatic disc 7.5–11 mm in diameter, crenate at margin with 7–14 rays; stamens 60–100 _____ *Nuphar* × *spenneriana*

5b. Leaves with 11–18 lateral veins on each side; stigmatic disc 6–8.5 mm in diameter, distinctly lobed at margin, with 7–12 rays; stamens 37–65 _____ *N. pumila*

## Acknowledgements

Many people have helped with this project; David Long in particular has been supportive and informative throughout. Andy and Liz Amphlett and Mark Duffell visited populations for us, and Fred Rumsey and Ian Green accompanied the first author during site visits. Don Padgett kindly responded to queries about the genus and commented on an earlier draft of this manuscript. The following all provided information on sites and records or suggested contacts: Carol Crawford, Alan Leslie, Dave Lang, Andy McVeigh, Michael Philip, Martin Rand, Gill Smart, Mark Spencer, Clive Stace, Jeff Waddell, Keith Watson and Sarah Whild. The authors are extremely grateful for their assistance. R.V.L. would also like to thank the owners of Shepperton Open Water Swim and Shropham Ponds for permission to access the sites. This project was partly funded by a research grant from the BSBI. The Royal Botanic


**Figure 10.** *Nuphar lutea* leaves: A, in July 2021 with a high proportion of emergent leaves, from Pop5 population, Carlingwark Loch, England; B, in August 2020 with a mixture of emergent and floating leaves, from Pop1 population, Ashtead Park, England; C, in July 2021 with submerged and floating leaves, from (no population code) Elterwater, England. Photographs: R. V. Lansdown.

Garden Edinburgh is supported by the Scottish Government's Rural and Environment Science and Analytical Services Division.

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