Microscopic features useful for identifying *Utricularia* native to the western USA, with an emphasis on the bladder quadrifid glands

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Introduction

Identifying species of *Utricularia* can be challenging, especially when the plants are not in flower. Even in the western USA, where relatively few species occur, a non-flowering mass of *Utricularia* shoots floating in the water can be difficult.

Of course, flowering *Utricularia* can be identified relatively easily. Out of flower, the patient student can identify species by a number of vegetative characteristics (shoot dimorphism, leaf segment flattening, and leaf setulae being key). However, when flowers are not present, or the collected specimen is incomplete, or the leaves are hopelessly mired in muck (as often the case on herbarium specimens) a confident identification can be challenging.

Fortunately, the bladders can be used to help in identifications. On the interior walls of the bladders one can find peculiar, four-armed glands that are called the quadrifid glands (Figs. 1A-F). Occasionally, one may also encounter two armed (bifid) glands (Fig. 1C). The glands are tiny—even the largest I have observed are less than 120 microns long—about 0.1 mm, so must be observed with a compound microscope at magnifications of 100-1000×. The function of these glands is a matter of contention, but probably is associated with removing fluid from inside the bladder, as well as carnivorous functions (Taylor 1989).

This paper is a primer on how to use the bladder glands to identify species in the western states of the USA, and is a useful supplement the botanical treatment I wrote for the California Flora (Rice 2012). Not only do the quadrifid glands have diagnostic features that are unique to each species, but these features can be used to identify both freshly collected, live specimens and also rehydrated herbarium samples.

Preparing specimens

Preparing *Utricularia* bladders for microscopy is a delicate process. Live or dead, the easiest bladders to work with are the largest you can find. Also, it is best if the bladders are as free of internal detritus or prey as possible. Extremely heavily pigmented bladders can also be difficult to see through—*Utricularia macrorhiza* bladders are sometimes purplish to nearly black, so look for younger bladders that have not yet pigmented. Live or dead, the specimens must usually be prepared under a dissecting microscope.

When I am examining live bladders, I usually quickly look at them under a compound microscope, under a cover slip, with no prior preparation—often the bladder glands can be seen with no further work. Otherwise, the nearly spherical bladder must be reduced to a specimen consisting of

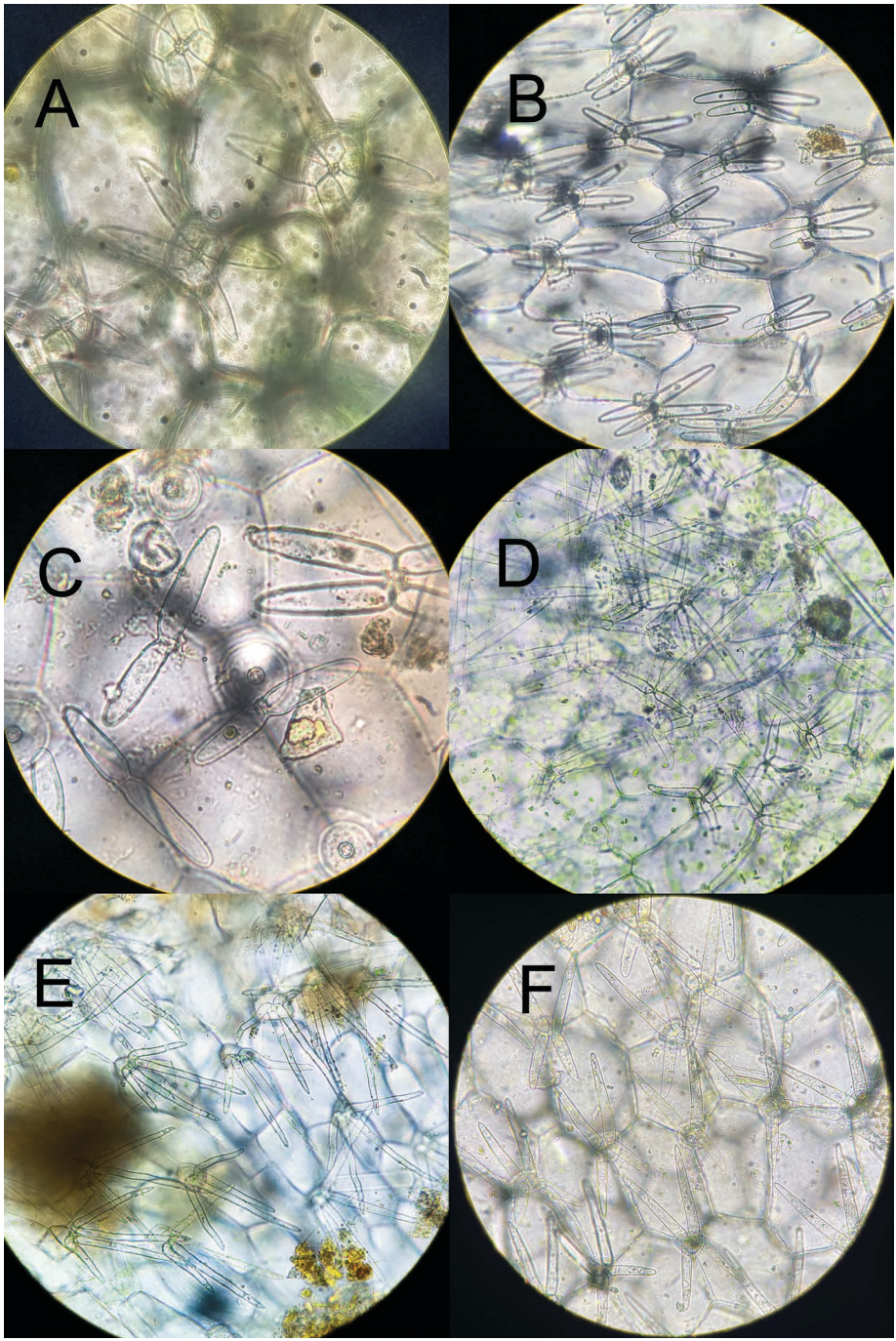

Figure 1: *Utricularia* glands. A: *U. gibba,* 1000×, Nevada County, California; B: *U. intermedia,* 400×, Butte County, California; C: *U. intermedia* (note the bifids), 1000×, Butte County, California; D: *U. macrorhiza,* 400×, Lassen County, California; E: *U. minor,* 400×, Lassen County, California; F: *U. ochroleuca,* 400×, Lassen County, California.

its flattened walls. If this is the case, use one or two needles to remove the trap opening by crushing, cutting, and tearing. Next, split the trap open, splaying the walls into two halves—a bladder fillet.

Despite the violence of this process, the majority of the quadrifid glands will retain their critical characteristics. Sometimes this preparation process is quite destructive, and only small portions of the trap walls survive the process…more than one bladder may have to be dissected. Once the specimens are prepared, they are placed under a coverslip.

When I am examining dried herbarium specimens, only a few bladders must be removed for examination. (Destructively sampling an herbarium specimen—even just removing a few bladders—requires permission from the owner of the specimen.) Sometimes loose specimen fragments are included on an herbarium sheet in an attached envelope, but even if not, usually a few loose bladders can be found here or there on the herbarium sheet.

Once the dried, flattened bladder has been removed, I look at it under a compound microscope, in case quadrifids can be seen with no further dissection. But if the bladder must be dissected, it can be using the method described as for live specimens. A different method, that I learned from Jan Schlauer, is to sandwich the bladder between two pieces of transparent tape. Then, by carefully separating the tape, each piece of tape carries away one separate bladder wall, ready for microscopy!

I examine the bladder specimen while it is still dry, then I moisten the specimen and examine it again. Almost invariably, in either dry or wet conditions, the quadrifid glands can be detected. While somewhat shriveled, their key characters can be observed. The results of my observations are always noted on the herbarium specimen as an annotation.

Key characters

Structurally, quadrifid glands usually have two pairs of oppositely directed gland arms. One pair is often consistently shorter than the other pair—hence the terms long arms and short arms. The two arms in a pair may be essentially parallel (diverging from each other by $0\text{-}30^0$) or by as much as 180^0. In some cases, the arms may diverge by even more than 180^0, so that both arm pairs are pointing in the same general direction.

While quadrifids may be useful in making identifications in combination with other observed features of a specimen, in the western USA, quadrifids by themselves can be used quite effectively to key a plant to species in nearly all cases. What follows is a key based purely upon quadrifid gland characters. When using this key, it is important to look at several examples of glands in a trap, and use the average or typical gland characteristics…it is always possible that a single gland may be distorted by the specimen preparation process.

1A: The angle between the short arms is 90° or more 2
  2A: The angle between the short arms is 90-180° 3
    3A: Short arms are less than ½ the length of the long arms; long arms are up to 120 microns long; long arms as much as 13× longer than wide ..... *U. macrorhiza*
    3B: Short arms are ½ to 1× the length of the long arms; the long arms are less than 50 microns long; long arms are 6-10× longer than wide ..... *U. ochroleuca* (1)
  2B: The angle between the short arms is 270-300° ..... *U. minor*
1B: The angle between the short arms is 0-80° 4
  4A: Long arms and short arms are in parallel pairs, diverging by 0-30° ..... *U. intermedia*
  4B: Long arms and short arms diverge by more than 30° 5

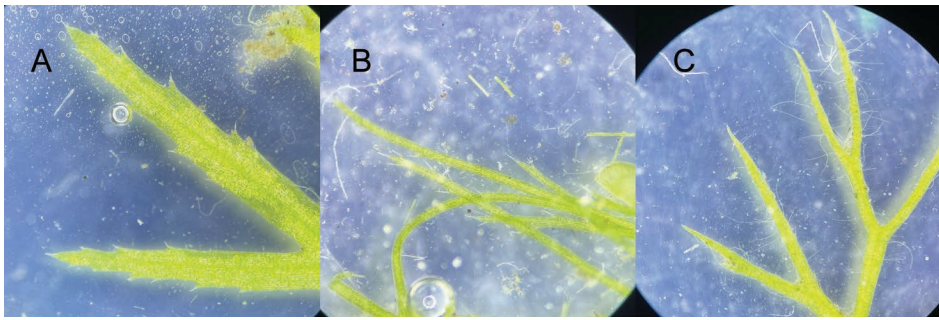

Figure 2: A: *Utricularia ochroleuca*; B: *Utricularia macrorhiza*; and C: *Utricularia minor* leaf segment tips at 45x. All specimens from Lassen County, California.

5A: All arms are approximately the same length; all arms are 1.5-6× longer than wide; both arm pairs diverge by approximately the same angle..... *U. gibba*
5B: A long and short arm pair dimorphism is clearly present; the long arms are 6-10× longer than wide; the long arm pair diverges by 20-45° while the short arm pair diverges by 40-160°..... *U. ochroleuca* (2)

This key is fairly straightforward, except for challenges with *Utricularia ochroleuca*, which has somewhat unremarkable glands. As such, couplets 3 and 5 above can be difficult to apply. Fortunately, even inadequate collections can easily resolve *Utricularia ochroleuca* from *U. macrorhiza*, and *Utricularia ochroleuca* from *U. minor* on other characters such as leaf morphology, shoot dimorphism, and bladder size. In particular, the leaves of *U. ochroleuca*, *U. macrorhiza*, and *U. minor* are distinctive. On the ultimate leaf segments (which are flattened), *Utricularia ochroleuca* bears numerous tiny spinelike hairs (setae) on the leaf margins, and the hairs are borne at the tips of tiny lateral teeth (Fig. 2A). In contrast, the ultimate leaf segments of *U. macrorhiza* are hairlike (not flattened), and the setae are larger but are not borne on tiny marginal teeth (Fig. 2B). Finally, the leaf segments of *U. minor* are at least slightly flattened, and almost never bear setae on the leaf margins (Fig. 2C) —although they occasionally are borne on the leaf segment tips.

Species comments

Utricularia gibba (Fig. 1A)

In my home state of California, this is a confounding species. It occurs at high elevations in the central Sierra Nevada (Tuolumne to Fresno Counties), and also at scattered coastal sites—these appear to be native populations. Meanwhile, additional populations appear sporadically in the Central Valley, especially in rice farming operations in Butte County. It is unclear if these populations are native or introduced, and how they are moving from site to site—either by agricultural equipment or waterfowl.

The quadrifid arms are small (10-40 microns long), with two pairs of more or less similar arms, weakly diverging. The arms are long-ovoid with acute tips. The bladders on this species are never large.

Utricularia intermedia (Figs. 1B, 1C)

This species produces its bladders on fragile, white or transparent shoots that affix the plant to the muck, and which require care to remove. Accordingly, hastily collected specimens often lack

bladders-bearing shoots. However, if they are present, the bladders—which are very large—are easy to work with.

The quadrifids arms are fairly small (approximately 50 microns long), with two pairs of nearly parallel arms. You will frequently see weakly diverging arms, but you will also see pairs of arms appressed tightly against each other—a feature you will never see in other species. The arms are uniformly wide over most of their lengths, and are usually blunt tipped.

Utricularia macrorhiza (Fig. 1D)

Very often, botanists not experienced with the genus trying to identify small plants will call it "*Utricularia minor*" based on stature alone, but under less than ideal conditions *Utricularia macrorhiza* can be quite small. Even dwarfed or stressed, the quadrifid gland morphology is reliable.

This species is usually very easy to work with. The dimorphism between arm pairs—long vs. short, and weakly diverging vs. strongly diverging—is easily visible. The angle between the short arms can be variable, as is seen in Figure 1D. The long arms are large (up to 120 microns long). The arms weakly tapering, and blunt tipped.

Utricularia minor (Fig. 1E)

Despite typically bearing small bladders, the quadrifids of this species are usually very visible. The quadrifid arms are large (about 100 microns), which is remarkable considering the bladders may be less than 1 mm (1000 microns) in size! The arms are very gently tapering, and acutely tipped.

Utricularia ochroleuca (Fig. 1F)

This species is generally considered to be of hybrid origin, and it is debatable whether it should be considered a true species, or simply a sporadic hybrid that is at best an intriguing curiosity. The plant is very similar to *U. intermedia*, but is often a little less robust. The quadrifid arms are moderately small (50 microns), are weakly tapering, with acute tips.

Note that Figure 3 is a rehydrated herbarium specimen, and despite having been dried, the critical quadrifid gland characteristics have been retained.

Work by Thor (1988) suggests that *Utricularia ochroleuca*, in the broad sense, is actually a mix of two species—*Utricularia ochroleuca* and *U. stygia*. Among the differences in these two species are subtleties in quadrifid structure. Schlosser (2003) used these differences to conclude that at least some of the populations of *Utricularia ochroleuca* in California should be considered *U. stygia*. Schlosser summarized the quadrifid glands of the two putative taxa as follows:

Utricularia ochroleuca: long arms separated by 18-52^0, short arms separated by 146-196^0. *Utricularia stygia*: long arms separated by 26-56^0, short arms separated by 52-96^0.

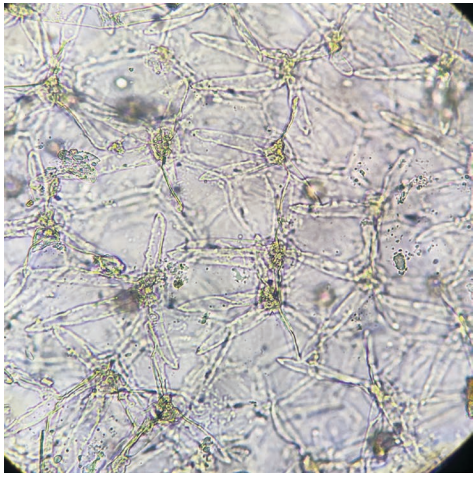

Figure 3: *Utricularia ochroleuca,* 400×. Rehydrated specimen, Teton County, Wyoming.

Generally speaking, *Utricularia stygia* quadrifids would have a structure more similar to *U. gibba*, while *U. ochroleuca* (more narrowly described) would look more similar to *U. macrorhiza*. Whether one considers these significant on a species level, or even if *U. ochroleuca* (broadly described) itself should be treated as a species, is a matter for discussions and fistfights.

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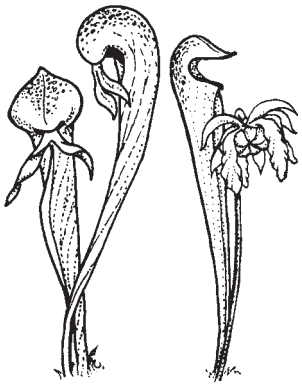

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Front Cover: *Utricularia minor*, Eldorado County, California, USA. Photo by Barry Rice. Article on page 194.

Back Cover: *Cephalotus* flowers at different stages from blooming to releasing seeds. Image by John Brittnacher. Article on page 168.

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