Reviewer 2

I find that my suggestions for improvement have been mostly incorporated into the revised paper; as I declared in the previous round, I consider the topic of this paper adequate and interesting to be published.

Finally, (as the authors exposed) further work with this species will be necessary to really understand the role of self-compatibility (S-morph), self-incompatibility (L-morph) and vegetative reproduction in the evolution and stability of both morphs in natural and invasive populations.

Reviewer 3

In this manuscript, the authors make a comprehensive description of the self-incompatibility system in Ludwigia grandiflora. The main results of the manuscripts are that Ldh has two floral morphs that differ in the length of the style and that these floral morphs have differences in SI system. Specifically, the L-morph has a late SI system, whereas the S-morph is self-compatible. The manuscript has interesting results based on laboratory work and fieldwork, which are noteworthy and deserve to be published. However, in its current form, the manuscript has some important inaccuracies, and some changes need to be made. Mostly, I think there are some misuses of terms that could create confusion in the literature pertaining to the evolution of floral polymorphisms. If these inaccuracies are addressed, the manuscript has the potential to be recommended.

I went through the manuscript (first) and then through the authors' response to the comments of the Reviewers. I, however, see that despite the thorough response to the previous Reviewer's comments, no changes were made in the original manuscript (or at least not in the one that was given to review [https://doi.org/10.1101/2021.07.15.452457]).

I attempt to provide constructive criticism, as aked by the authors, in the hope that the authors reconsider and incorporate the comments of previous Reviewers that could improve ths manuscript. I will provide comments on the response from the authors (first) and then give specific comments on the current manuscript as it is now.

Specifically, I agree that Ludwigia grandiflora does not have heterostyly. Although there is a difference in the style length among the floral morphs (supported by statistical analyses in this manuscript), this floral system may be considered a style dimorphic species (with approach and reverse herkogamy). This is because the distinctive character of heterostyly is not the difference in style length alone, but the case of reverse herkogamy. Therefore, differences in anther position and the measure of herkogamy should also be reported (as pointed out by Reviewer 2) if one is to study heterostyly.

In their response to Reviewer 1 (from now on R1), the authors indicate that the main aim of the manuscript is to identify the mating system of Ldh and not about invasion ecology. I agree with this. However, the authors stress invasion too much in the Introduction that may mislead the reader into thinking that the paper is about invasion ecology (which also happened to me). I would temper the use of invasion in the Introduction keep it in the Discussion section. In fact, the observation that the SI morph is the most common in invasive populations is intriguing that could be exploited in the Discussion rather than in the Introduction.

Given that a great deal of the manuscript is based on the idea of having different floral morphs, I disagree with the authors in the part that "we don't interest in heteromorphy and floral biometry" and in "Whatever, whether or not this plant is heteromorphic is not the purpose of this current manuscript and definitely doesn't matter to study.". The authors say, "If specialists can demonstrate this is not heteromorphy or something else, we would be interested to read the demonstration and explanation, out of an argument of authority about a reference that reviewed ... ". My assessment of the paper (and the reviews) is not that Ldh does not have heteromorphy (which it has). Still, this heteromorphy (i.e., floral dimorphism) is NOT heterostyly. In fact, I don't believe that R1 and R2 disagree in that there are two floral morphs. They, however, disagree with the misuse of the term heterostyly. Heterostyly has a precise definition (Barrett 2019). I think that the authors demonstrate, very convincingly, that the flowers of Ldh are dimorphic, and thus, the authors can keep their use of L- and S-morph if they define them as approach and reverse herkogamy, but not as heterostylous. As indicated by the authors, the elimination of the term 'heterostyly' will not change the results; rather, it will keep consistency in the literature of heterostyly. Many problems in the evolution of floral polymorphisms occur due to misuse of these terms. In fact, one part of the Discussion could address whether Ldh is heterostylous or not. Moreover, one of the interesting results is that different floral morphs are associated with different SI systems, and if this is an original observation from this manuscript, the authors should exploit this in the Discussion.

I agree with R2 in that the possibility of vegetative reproduction should be at least acknowledged as an explanation of the fact that the SI morph (L-morph) is the most common in invasive populations. Authors do not need to make a whole discussion about it but only indicate that. Maybe supporting information from related taxa could be helpful.

In general, it would be valuable if the authors acknowledge the limitation of their study, in Discussion, and propose experiments that could fill the gaps in our knowledge regarding the reproduction Ldh and the role of reproduction in invasive population of Ldh.

Line 50: Get rid of "and beyond, and understand their phylogeny and evolution" since it adds nothing to the sentence and it is unclear. For instance, what is "beyond" angiosperms? It is unclear if the part that says 'their phylogeny' refers to angiosperms or to SI systems. In either case, SI is not essential to understanding the phylogeny of angiosperms. Moreover, one is interested in the 'phylogenetic distribution' of SI, not its phylogeny. I understand that the authors do not want to debate 'semantics' and 'terminology,' but some level of consistency should be kept for the scientific literature in order to avoid future confusion.
Line 42: Mixed mating system is misused here and in several parts of the manuscript (Lines: 6, 42, 330, 419, and 423). Although there seem to be potential differences in the mating system among the population (and maybe even within), the mating system requires specific sets of experiments (pollen flow among individuals, estimation of outcrossing rates, etc.). Mixed mating systems are defined as outcrossing rates between 0.2 and 0.8 (Goodwillie et al., 2005).
Line 58: I think that the use of 'peripatric' is very specific in this part of the manuscript, so I would not use it here.
Line 60: The authors already defined self-incompatibility as SI in the first paragraph. Use SI consistently throughout the manuscript, e.g., Line 359 and 363and so on (except when one starts a sentence).
Line 66: The authors define HetSI but then never use this acronym again. I would eliminate

this acronym.
Line 69: The part "spatial distancing of the anthers and stigma in the 3D architecture of a flower" says nothing about heterostyly. By eliminating the introduction about heterostyly, the authors can get rid of this part.
Line 73: add a comma after 'i.e.' as is done in other parts of the ms.
Line 87: It is unclear to me why the use of 'literally' is important here.
Line 91: Ludwigia needs to be italicized.
Line 93: Change "Water primrose ..." to "The water Primrose ..."
Line 95: It would be great to know where does Ldh comes from (place of origin) and where it is invasive.
Line 99: This is where the confusion is generated. Ldh is already defined as heterostylous in the manuscript based on Portillo-Lemus et al. (2021) when in fact, this has not been proven (see my comment above). In fact, the term used in that paper is "heteromorphic reproductive system, " which is correct. Thus, I would suggest changing heterostyly to floral heteromorphism in all sections when discussing Ldh (e.g., Lines 346, 348, and 351 in discussion). Here the authors could define more clearly what they indicate as S- and L-morph.
Line 135: Eliminate "... compared the results we obtained with other species, especially from the Myrtales order to ... ". the comparison is irrelevant for the sentence.

Methods:

Line 215: Add space between the two paragraphs.

Results:

Line 252: Whenever p-value =<10-15 just state p-value <0.001. If it has 10, 13, or 15 zeroes, it makes no difference.
LIne 258: "... and whatever the pollen origin." could be changed to "... independent of the pollen origin".
Line 324: There seems to be a dash above a dot. Possibly due to a track change in Word.

Discussion:

Line 330: The term of mixed mating system is misused. Mixed mating indicates that outcrossing rates are between 0.2 to 0.8 (Goodwillie et al., 2006).
Line 335: The use of 'literally' is unnecessary.
Line 340: Change 'all self-pollens' to 'all self-pollen grains'.
Line 370: Vochysia should be in italics.
Line 377-385: It would be good if the authors point out the necessary experiments to determine 'reproductive assurance' in Ldh. For example, bagging experiments.

Table S1: In 3rd column it says 'fruitless' for some L-morph populations but then the fruit set is presented in the following columns which is contradictory information.

Figure S1: There are two 'S-morph self' treatments. I wonder if one of them is meant to be 'L-morph self'.

Reviewer 1

Dear PCI Ecology recommenders,

After reading the rebuttal letter by LO Portillo Lemus et al to my former review of their manuscript “Late-acting self-incompatible system, preferential allogamy and delayed selfing in the heterostylous invasive populations of Ludwigia grandiflora subsp. hexapetala” I consider that some of the most critical concerns I raised are still unresolved in their letter.

First, I would like to apologize if they feel that my review provoked a rude and hurried (their words) rejection or, more properly, an initial lack of recommendation. It was not my aim such a rudeness nor a rapid review. I read the manuscript several times and went to the relevant literature around the topic, including former published (that is, open to public scrutiny) work by the authors. It is my regular way to review.

Yes indeed, I consider the topic interesting of course. I have some doubts about its fitting to the scope of PCI Ecology, but this is subject to opinion which Recommenders and Managing Board should resolve, of course. My concerns were not about the semantics or the terms, and it is not a matter of being botanist, ecologist, or evolutionist. If I was very detailed in my report it was because I usually do that. I am or have been editor in other journals and know how frustrating can be a rejection based only in a short paragraph by an “established” reviewer. Although I have met similar cases as an editor, it is not common at all to re-submit the same manuscript after clear rejection. They have not corrected even those items where they offer a solution to concerns raised by at least one of the reviewers. It would have been good if at least these were addressed in a new version. This means that they prefer to put all time and effort in discussing the validity of the manuscript on very general grounds and opinions about the review process, which I think it is not the right place to do.

My main concerns were about the sampling design (how to know they were sampling different genotypes within a single highly clonal species is critical for this kind of study) and about stamen measurements, which are lacking and are necessary to determine heterostyly. If they do not want to speak about heterostyly, it is very easy to solve, just avoid it, from the very starting point: the title. This proper sampling of different genets is not addressed at all in their rebuttal letter. In fact, they answer to one of my concerns about this issue:

“Lines 169-179. The numbers of samples in this paragraph (which are large indeed) does not refers to how many individual plants (genets) and this is critical.

Answer: why it is critical knowing of randomly-sampled individuals in populations of thousands of plants are clones (ramets) or genets to understand their self-(in)compatiblity and if their selfed and outcrossed seeds are viable?”

Even if they aim to address in their study only incompatibility system, it is completely necessary to work with different genotypes. Incompatibility types, groups or morphs, if they prefer, are genetically based, thus the same genotype belong always to the same group. Since this is a problem of the design, I do not see how is possible to solve without a new sampling, or much easier, with a genetic screening of the plants used just to determine they are not clones. Random sampling of plants is fine for annual or perennial plants with no vegetative reproduction, simply by sampling a few meters apart; but this does not seem to be the case.

Just to be positive about the destiny of the manuscript, I prefer not to participate in the recommendation process further. I still think that there is an interesting point in their study (late acting self-incompatibility, in which I am not an expert). Finally, I have no objections, again, to accept a new case of heterostyly, when properly measured and analyzed. There are some recent cases in the literature which authors can check. This new case would much help in explaining the evolution of this breeding system, which usually provokes an outcrossing mating system in most of populations bearing it. I sincerely wish the authors are successful in their pursuits.