



Wrocław, June 22, 2017

Dear Editors and Reviewers,

Encouraged by your letter, we would like to re-submit the manuscript entitled:

**Leaf arrangements are invalid in the taxonomy of orchid species** by A. Jakubská-Busse, E. Żołubak, Z. Łobas and E.M. Gola.

We are very much thankful to the Reviewers for their deep and thorough reviews. We have revised our paper according to their useful suggestions and comments. All specific comments are addressed in the revised version of the manuscript; all changes are marked in red. We hope that you will find this revised and improved version of the manuscript suitable for publishing in the *PeerJ*.

Thank you once again for considering the publication of our manuscript in *PeerJ*.

Yours sincerely,

On behalf of all authors,

Anna Jakubská-Busse

### ***Response to Reviewer #1 Comments***

#### **Reviewer #1: Basic reporting**

*In this study the authors examined a specific phenotypic trait (leaf arrangement) in an orchid species belonging to the taxonomic complex genus Epipactis. Specifically, authors, through a morphological survey of herbarium specimen and natural populations demonstrated the absence of the distichous phyllotaxy in this species. They also showed the presence of an atypical decussate phyllotaxis and the ontogenetic formation of pseudowhorls. The authors conclude that their data demonstrate the great ontogenetic variability and plasticity on E. purpurata. The Manuscript is well written and data are well analysed and presented but I have major concerns about the premises and the relevance of the study.*

#### **and Experimental design**

*Authors start from the consideration that the limited availability of molecular tools make it very important the identification of morphological traits for orchid classification. In this context I would have cited the paper from Tranchida et al. 2010 on molecular tools for species delimitation in Epipactis.*

*They also noticed that several traits as the column are highly indicated for this purpose because they can be in some way related to reproductive isolation and speciation. However, they focus on a trait that is in reality not very useful for species delimitation and that for sure has no evolutionary implications. Indeed, they found that it is inconsistent within a species.*

**Response: Thank you for these comments. We fully agree that the molecular tools are used for the modern phylogeny of orchids and *Epipactis* in particular. However, there is still a question about the correctness of the identification of plant material for genetic analyses which is mostly**

validated based on morphological traits. In addition, revisions of the plant collections, including herbarium vouchers, are performed based on morphological features and have to be done without the damage to the material; only in particular cases the sampling of the voucher material is allowed. Thus in this paper we rather aimed to increase the awareness especially of field-researchers who often need to identify plant material in situ, and when sometimes only young non-flowering yet plants are available about phenotypic plasticity in the genus *Epipactis*. We added such explanation to Introduction.

**Reviewer # 1:** *Validity of the findings*

*Overall the authors showed that there is a great ontogenetic variability in the investigated traits. I fear that this finding was highly predictable and that results are not useful for the stated purpose of identifying distinguishing characters among orchid species.*

*Authors state that “the type of leaf arrangement is even used to distinguish between or to characterise complex taxa, for example, *E. atrorubens*, *E. tremolsii* and *E. helleborine*”. This is mainly not true basing on my sources as the three cited species are mainly defined on the basis of leaf traits other than their disposition. Also these species are even not universally accepted as species (see world monocot checklist). Even admitting that leaf arrangements would be an important trait for species categorization, they focus on a species *E. purpurata* that is generally identified basing on other traits.*

**Response:** That is true that the species diagnostic features (also for those species mentioned above) include mostly other features than the leaf arrangement but unfortunately in the popular manuals they appear sometimes as dichotomizing traits within complex taxa; e.g. in Delforge 2006, in *E. atrorubens* group, distichous vs. spiral leaf arrangement is given at the second level of distinguishing features after leaf length vs. internode but prior to flower traits. This shows that vegetative traits are also utilized during the dichotomous key construction and thus also during plant identification. Aware of plant plasticity and developmental changeability we wanted to pay attention to these phenomena. Furthermore, the proper leaf arrangement has not been studied in this group of orchids, and theoretically based on manuals both leaf arrangements spiral and distichous, with different frequencies, could occur. In addition, leaf arrangement can erroneously be identified as distichous when leaves are separated and distant one to another at the elongated stem; then the ontogenetic spiral is stretched and phyllotaxis is not obvious. Thus, even and maybe especially in the species so-well defined based on morphological traits as *E. purpurata* the occurrence of developmental and intraspecific variability is even more interesting.

**Reviewer # 1:** *Comments for the author*

*In this study the authors examined a specific phenotypic trait (leaf arrangement) in an orchid species belonging to the taxonomic complex genus *Epipactis*. Authors claim that leaf arrangement is an important trait for species*

categorization but concluded that this trait cannot be used because it is inconsistent.

The Manuscript is well written and data are well analysed but I have major concerns about the premises and the relevance of the study.

Below are my main criticisms:

Authors start from the consideration that the limited availability of molecular tools make it very important the identification of morphological traits for orchid classification but they overlook studies as Tranchida et al. 2010. They also noticed that several traits as the column are highly indicated for this purpose because they can be in some way related to reproductive isolation and speciation. However, they focus on a trait that is in reality not very useful for species delimitation and that for sure has no evolutionary implications. Indeed, they found that it is highly variable within species.

Authors state that "The type of leaf arrangement is even used to distinguish between or to characterise complex taxa, for example, *E. atrorubens*, *E. tremolsii* and *E. helleborine*". This is mainly not true and these species are even not universally accepted as species (see world monocot checklist) and are generally described using parameters other than leaf arrangements. Even admitting that leaf arrangements would be an important trait for species categorization, they focus on a species *E. purpurata* that is generally identified basing on different traits. In this context, their findings were widely predictable.

**Response:** We corrected the text according to the comments, adding information on the usage of the molecular tools in *Epipactis* taxonomy and explaining why vegetative features are still important in orchid classification. The most importantly we rephrased the conclusion on the usefulness of leaf arrangement for distinguishing the aggregates of *Epipactis*. The comments referring to the predictability of our results have been addressed above.

### ***Response to Reviewer #2 Comments***

**Reviewer Climbiê Hall:** *Basic reporting*

*There is some parts of the text that need additional references.*

*There is only one reference from the past four years, and not a lot more from this decade. I think that the authors should search for more recent literature to discuss to show that this kind of study still important.*

**Response:** As suggested, some additional more recent references have been added to the text.

**Reviewer Climbiê Hall:** *The last figure should be colored to improve clarity.*

**Response:** As suggested, the last figure is presented in color in the corrected version of the paper.

**Reviewer Climbiê Hall:** *The last goal of the study was not discussed enough.*

**Response:** In the revised version, we moved the sentences about the differences in phyllotaxis between taxa from Introduction to Discussion and rephrased the text. We aimed to prove that the ontogenetic and intraspecific variability of the leaf arrangements discussed in the text in general is a normal developmental phenomenon and it is also present in *Epipactis* (exemplified by *E. purpurata*).

**Reviewer Climbiê Hall:** *Experimental design*

*A statistical test would corroborate the discussed results, because the numbers in the results seem very low to support the conclusions.*

**And** *Validity of the findings*

*The authors have background to discuss about the genus as a whole and they stated that it was one of the study goals. Therefore, I think that this topic should be expanded in the discussion, improving the papers relevance. A statistical test would corroborate the discussed results, because the numbers in the results seem very low to support the conclusions*

**Response:** We thank for these comments. We formulated our conclusion in the way it could be confusing which we were not aware of. We meant that the spiral leaf arrangement is a typical dominant pattern in *Epipactis* and so sporadic occurrence of decussate phyllotaxis (<1%) does not have taxonomic significance. The feature itself is binary (present-absent), qualitative not really the quantitative, and differences between the occurrence of either of the conditions are overwhelming. Therefore, we did not perform the statistical tests. However, we rephrased the conclusion driven based on these numbers to make it clear, as you suggested.

**Reviewer Climbiê Hall:** *Comments for the author*

*The paper is interesting, but should be improved. A broader discussion about the genus is needed and the conclusions seem exaggerated. Most of my suggestions are in the pdf file accompanying this review.*

**Response:** We are grateful to the Reviewer for encouraging comments in the PDF file. We changed parts of the text accordingly and softened the conclusions.