**Simple verification of *in vitro*-grown clones of the genus *Drosera* L. using ITS molecular markers**


## Abstract

*Drosera* L. is a genus of carnivorous plants that comprises approximately 250 species, although this number is probably not complete. Some of these taxa exhibit only small differences in morphological traits that can be partly influenced if the taxa are propagated *in vitro*. Here, we focus on the verification of putative clones of *Drosera spathulata* Labill., *Drosera rotundifolia* L. and *Drosera binata* var. *Dichotoma* species cultivated *in vitro* using molecular markers covering the internal transcribed spacer (ITS) region of 45S ribosomal DNA (rDNA). Following the polymerase chain reaction (PCR) amplification of ~360-bp DNA fragments and sequencing, the sequences were aligned with corresponding sequences in the National Center for Biotechnology Information (NCBI) database. In addition, each of tested PCR amplicons had a specific restriction profile that predominantly enables the differentiation of *D. rotundifolia* and *D. spathulata*; the shape of the leaves does not have to be a clear morphologically distinguishable trait.



Key words: *Drosera binata*, *Drosera rotundifolia*, *Drosera spathulata*, ITS nucleotide sequences, molecular identification


## Introduction

Currently, chloroplast (*rbc*L) and ribosomal (internal transcribed spacer (ITS)) DNA sequences are extensively employed in molecular studies aimed at determining inter- and intrageneric relationships in evolving taxa, as these sequences have high mutation rates (Williams et al. 1994; Baldwin et al. 1995; Fineschi et al. 2002; Rosato et al. 2016). The *rbc*L analysis of ~100 plant species including families of the subclasses Rosidae, Hamamelidae, Nepenthaceae and Caryophyllidae placed monophyletic Droseraceae in the same clade as Caryophyllidae and Nepenthaceae. Combined *rbc*L and 18S ribosomal DNA (rDNA) sequence data were then used to infer phylogenetic relationships among the genera of the Droseraceae family, in which three of four genera, *Drosera*, *Dionaea* and *Aldrovanda*, exerted monophyletic character (Rivadavia et al. 2003). Moreover, molecular analyses clustered most of the taxa of the *Drosera* L. genus into the *regia* clade, which contains *Drosera regia*; the *capensis* clade, which involves the South

**Commented [1]: Issue:** The title emphasizes "simple verification" which may understate the methodological rigor and novel application of ITS markers for in vitro clone identification, reducing perceived impact. **Suggestion:** Revise the title to highlight the novel combination of ITS sequencing and restriction profiling for resolving taxonomic uncertainties in morphologically plastic in vitro clones, e.g., "Molecular Verification of In Vitro-Grown Drosera Clones Using ITS Sequencing and Restriction Profiling."

**Commented [2]: Issue:** The title includes "Drosera binata var. Dichotoma," but the Introduction and subsequent sections refer only to "Drosera binata" without specifying the variety, creating inconsistency. This omission may confuse readers about the exact taxonomic scope of the study. ... [1]

**Commented [3]: Issue:** The title does not clearly state the main conclusion or novel aspect of the study, such as the development of restriction profiles for differentiation, which limits its ability to convey the ... [2]

**Commented [4]: Issue:** The abstract's opening sentence mentions the incomplete species count but does not tie this to the central problem of taxonomic uncertainty in cultivated clones, missing an opportu ... [3]

**Commented [5]: Issue:** This sentence begins the abstract with general background about Drosera species diversity but does not establish the specific motivation or knowledge gap (e.g., challenges in verifying clones ... [4]

**Commented [6]: Issue:** This sentence introduces the study's focus but does not explicitly connect the need for molecular verification to the broader challenge of morphological plasticity in vitro, which is critical for ... [5]

**Commented [7]: Issue:** The aim statement in this sentence is overly technical and does not explicitly frame the study's purpose (e.g., resolving taxonomic uncertainty in clones). ... [6]

**Commented [8]: Issue:** The statement about restriction profiles enabling differentiation of D. rotundifolia and D. spathulata lacks a clear link to practical applications, such as how this method improves efficiency over ... [7]

**Commented [9]: Issue:** The abstract's results section lacks quantitative data (e.g., sequence identity percentages or restriction fragment sizes) to support claims of differentiation between species. ... [8]

**Commented [10]: Issue:** The keywords omit critical terms like "restriction profiling" and "clonal verification," reducing discoverability. **Suggestion:** Revise keywords to include "restriction ... [9]

**Commented [11]: Issue:** This sentence lists multiple citations for the utility of ITS and rbcL markers but does not explicitly state why ITS was prioritized over rbcL or other markers for this study. The rationale for sele ... [10]

**Commented [12]: Issue:** The discussion of rbcL analysis in Droseraceae phylogeny is overly detailed and tangential to the study's focus on verifying clones using ITS markers. This distracts from the central narrati ... [11]

African and temperate species outside of Australia; and the *peltata* clade, which contains Australian endemics (Williams et al. 1994).

The majority of *Drosera* species have active fly traps and capture their prey with mobile glandular hairs that are present on the adaxial leaf surface (Rivadavia et al. 2003). Obovate- or spathulate-shaped leaves constitute a morphologically distinct trait of most of these taxa. Therefore, other characteristics such as the number of styles and the presence/absence of both stipules and specialised organs (tubers and gemmae) have been used for taxonomic classification. Later, chromosome number (Kondo et al. 1976), pollen morphology (Takahashi and Sohma 1982) and seed germination type (Conran et al. 1997) were also used to indicate the specificity of individual species. Most recently, DNA analyses including randomly amplified polymorphic DNA and the ITS region of the 18S-5.8S-26S nuclear ribosomal cistron have been used to identify nuclear genetic variation of individual species. In the case of 45S rDNA, the genetic regions are highly conserved, but ITS divergence is sufficient for resolving individual species within most genera (Matyášek et al. 2012).

In this work, we used ITS molecular markers for the verification of putative *in vitro* clones of *Drosera spathulata*, *Drosera rotundifolia* and *Drosera binata* species. In addition, for each of tested taxa, we proposed a specific restriction profile of amplified ITS polymerase chain reaction (PCR) products.

## Materials and methods

### Plant material and DNA isolation

Plants of *D. binata*, *D. rotundifolia* L. and *D. spathulata* Labill. were provided as gifts from the Institute of Experimental Botany, Czech Academy of Sciences and cultivated aseptically on agar media as described previously (Bobak et al. 1995). Total genomic DNA was isolated from the fresh leaf material (1 g) of investigated taxa in accordance with the methods of Bekesiova et al. (1999).

### PCR amplification and sequencing

PCR fragments were amplified using the primers DribFOR (5′-AACCCCGGCGCAAGTCGCGCCAAGGA-3′) and DribREV (5′-AGCTTCCCATTTGGGCCAACCGCG-3′) designed for the sequences covering the 18S ribosomal RNA (rRNA), ITS1, 5.8S rRNA, ITS2 and 28S rRNA, accounting for the availability of overlapping sequences of analysed species stated in National Center for Biotechnology

**Commented [13]: Issue:** The description of Drosera clades lacks a direct connection to the study's aim. Readers may struggle to see how clade classification relates to verifying in vitro clones, creating a narrative disconnect.
**Suggestion:** Before this sentence, introduce a statement explaining how clade-level genetic divergence informs the need for precise molecular tools to distinguish closely related species or clones.

**Commented [14]: Issue:** The discussion of fly traps and glandular hairs is irrelevant to the study's focus on molecular verification. This deviation disrupts the logical flow and fails to advance the rationale for using ITS markers.
**Suggestion:** Remove or replace this sentence with a discussion of morphological traits (e.g., leaf shape ... [12]

**Commented [15]: Issue:** This sentence describes leaf shape as a "morphologically distinct trait," which contradicts the Abstract's assertion that leaf shap ... [13]

**Commented [16]: Issue:** The mention of RAPD and ITS markers does not clarify why ITS was chosen over RAPD for this study. The absence of a comparative ration ... [14]

**Commented [17]: Issue:** This sentence states that ITS divergence resolves species within genera but does not address its applicability to clonal verification, whic ... [15]

**Commented [18]: Issue:** The sentence states the use of ITS markers but does not contextualize why ITS was selected over other markers (e.g., rbcL) for this spe ... [16]

**Commented [19]: Issue:** The aim statement lacks a clear articulation of the knowledge gap—specifically, why verifying in vitro clones of these species is nov ... [17]

**Commented [20]: Issue:** This sentence states the use of ITS markers but does not justify why ITS was selected over other markers (e.g., rbcL) for this specific ... [18]

**Commented [21]: Issue:** The mention of "proposed a specific restriction profile" is abrupt and lacks justification for why restriction analysis was priorit ... [19]

**Commented [22]: Issue:** The final sentence of the Introduction mentions restriction profiles but does not emphasize the broader significance of the findings ... [20]

**Commented [23]: Issue:** The origin of plant material is described as "provided as gifts," which lacks critical details such as the number of clones analyzed, the ... [21]

**Commented [24]: Issue:** The origin of plant material is described as a gift, but there is no mention of voucher specimen deposition in a herbarium or seed bank, ... [22]

**Commented [25]: Issue:** This sentence cites cultivation methods from a previous study but does not provide essential details about the in vitro growth conditio ... [23]

**Commented [26]: Issue:** this sentence does not specify the number of clones analyzed per species or replication details, raising concerns about experimental rigor. ... [24]

**Commented [27]: Issue:** This sentence references a DNA isolation protocol but lacks technical details (e.g., whether a commercial kit or CTAB-based method ... [25]

Information (NCBI) database (AB355664.1, HM204879.1 and AB685414.1). The PCR conditions were one cycle at 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 55 °C for 30s and 72 °C for 45 s; and a final elongation step at 72 °C for 10 min. The DNA amplified by PCR was cloned into a pGEM-Teasy (Promega, USA) vector and sequenced using an ABI Prism 310 genetic analyser with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA).

**Bioinformatic analysis of sequencing data**

Nucleotide sequences of the 18S-5.8S-26S rRNA genes of *D. rotundifolia* L. (AB355664.1), *D. binata* (HM204879.1) and *D. spathulata* (AB685414.1) were collected from the NCBI GenBank database.

The nucleotide sequences of the PCR amplicons of the analysed species cultivated *in vitro* were aligned with the rDNA sequences of the corresponding species using the CLUSTAL O v1.2.4 program (Thompson et al. 1994). PCR fragment restriction analysis was then performed using the NEBcutter v2.0 program (http://nc2.neb.com/NEBcutter2/). The identities of the studied taxa were also confirmed using the *Eco*RI, *Bgl*I and *Sph*I restriction enzymes.

**Results and discussion**

The verification of the identity of individual species using DNA molecular analysis is a crucial step for the genetically related individual *Drosera* species that have similar morphological characters (Tungkajiwangkoon et al. 2016) that may be slightly altered from *in vitro* cultivation (Batagin et al. 2009; Trejgell et al. 2012). In our case, this type of molecular verification precedes the isolation and expression profile characterisation of other genes of these poorly explored carnivorous species.

The 364-bp- and 368-bp-long fragments covering the sequences of ITS1 of the 5.8S and ITS2 of 45S rDNA, respectively, of *D. spathulata*, *D. binata* and *D. rotundifolia* were amplified using the DribFOR and DribREV primers. The design of primers for use in relatively conserved regions was limited by the availability of overlapping sequences in all tested plant species. After PCR, the DNA amplicons of the putative *D. rotundifolia*, *D. spathulata* and *D. binata* clones were TA cloned and sequenced. The alignment of obtained sequences with the corresponding sequences of *D. rotundifolia* (AB355664.1), *D. spathulata* (AB685414.1) and *D. binata* (HM204879.1) retrieved from the NCBI database confirmed the identity of the analysed *in vitro* clones.

**Commented [28]: Issue:** The primer design rationale is incompletely explained, as the sentence does not clarify how conserved regions were identified or why overlapping sequences were prioritized.
**Suggestion:** After this sentence, add a sentence describing how primer regions were selected based on alignment of conserved 18S/28S rRNA regions flanking the ITS segments across Drosera species.

**Commented [29]: Issue:** The primer sequences include non-standard notation (e.g., "5´-" instead of "5'-") and lack information about their synthesis (e.g., vendor, purification grade), which affects reproducibility.
**Suggestion:** In this sentence, correct the primer notation to "5'-" and specify the vendor (e.g., Integrated DNA Technologies) and purification method (e.g., HPLC-grade) for clarity.

**Commented [30]: Issue:** This sentence describes primer design but does not justify why these specific primers were selected over alternatives or how they were validated for specificity across the three Drosera species, which is critical for methodological rigor.
**Suggestion:** After this sentence, include a brief rationale for primer selection (e.g., alignment of conserved ... [26]

**Commented [31]: Issue:** This sentence describe ... [27]

**Commented [32]: Issue:** The PCR conditions om ... [28]

**Commented [33]: Issue:** The sequencing metho ... [29]

**Commented [34]: Issue:** This sentence states th ... [30]

**Commented [35]: Issue:** This sentence lists NCB ... [31]

**Commented [36]: Issue:** The alignment software ... [32]

**Commented [37]: Issue:** The use of NEBcutter v2 ... [33]

**Commented [38]: Issue:** The use of EcoRI, BglI, a ... [34]

**Commented [39]: Issue:** The Materials and Meth ... [35]

**Commented [40]: Issue:** This sentence names ... [36]

**Commented [41]: Issue:** This sentence mentions ... [37]

**Commented [42]: Issue:** This section combines ... [38]

**Commented [43]: Issue:** The Results section lac ... [39]

**Commented [44]: Issue:** The Results and Discus ... [40]

**Commented [45]: Issue:** This sentence begins th ... [41]

**Commented [46]: Issue:** This sentence cites pre ... [42]

**Commented [47]: Issue:** The reference to future ... [43]

**Commented [48]: Issue:** The mention of future g ... [44]

**Commented [49]: Issue:** This sentence describe ... [45]

**Commented [50]: Issue:** The description of PCR ... [46]

**Commented [51]: Issue:** This sentence reports ... [47]

**Commented [52]: Issue:** This sentence explains ... [48]

**Commented [53]: Issue:** This confirmation of clo ... [49]

**Commented [54]: Issue:** The alignment results a ... [50]

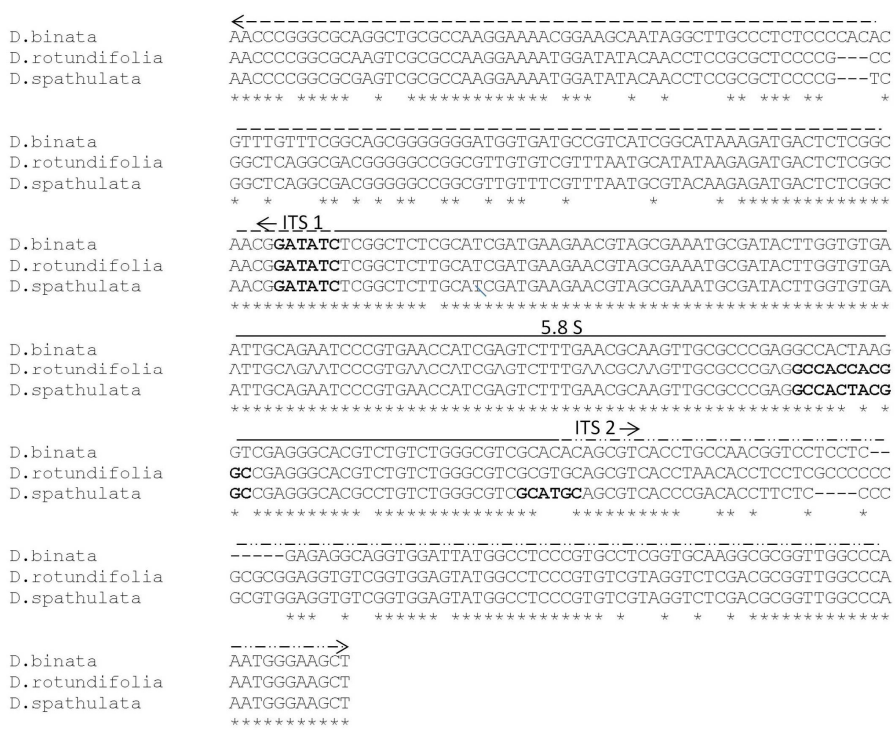



Fig. 1 Ribosomal DNA alignment of *Drosera binata* (HM204879.1), *Drosera rotundifolia* (AB355664.1) and *Drosera spathulata* (AB685414.1) intervening the nucleotide sequence between the DribFOR and DribREV primers. In *bold* are shown the restriction sites GATATC, GCCNNNNNGGC and GCATGC, which correspond to *Eco*RV, *Bgl*I and *Sph*I restriction endonucleases, respectively.

The occurrences of substitution mutations and indels within the analysed ITS sequences of these taxa were subsequently analysed. The regions of high homogeneity occurred mostly in the regions belonging to the 5.8S rDNA (coding part of rDNA units), as in most angiosperms, indicating strong selection pressure imposed on functionality (Rosato et al. 2016). In contrast, a higher level of variability was observed in the regions corresponding to ITS1 and ITS2 (Fig. 1). Among the analysed species, *D. binata* and *D. spathulata* shared a higher genetic divergence (88% identity and 3% of indels) than did *D. spathulata* and *D. rotundifolia* (96% identity and 1% of indels). This is in agreement with the results of previous phylogenetic studies that involve morphological as well as *rbc*L and 18S rDNA data (Williams et al. 1994; Rivadavia et al. 2003;

**Commented [55]: Issue:** The analysis of substitution mutations and indels is presented as observational data without connecting to the central theme of clone verification, missing an opportunity to discuss how sequence variability informs identification reliability.
**Suggestion:** In this sentence, explicitly state that the observed mutation patterns confirm ITS regions' suitability for discrimination between these species despite their close relationship, directly supporting the verification protocol's robustness.

**Commented [56]: Issue:** The analysis of substitution mutations and indels is introduced without stating the objective of this analysis, making it unclear how it relates to the study's aim.
**Suggestion:** Add a sentence before this sentence clarifying the purpose (e.g., "To assess genetic variability among the taxa, we analyzed substitution mutations and indels in the ITS sequences").

**Commented [57]: Issue:** This sentence cites Rosato et al. (2016) to explain evolutionary pressure, which is interpretive and belongs in the Discussion. The Results should avoid citations to prior work.
**Suggestion:** Remove the citation and rephrase to focus on the observed data (e.g., "High homogeneity in 5.8S rDNA suggests strong functional conservation").

**Commented [58]: Issue:** The observed ITS variability is attributed to general angiosperm patterns but not specifically linked to Drosera's unique biology as carnivorous plants with specialized morphology.
**Suggestion:** In this sentence, propose that high ITS variability in Drosera may reflect rapid adaptive evolution in carnivorous structures, connecting molecular findings to the genus' ecological specialization.

**Commented [59]: Issue:** The genetic divergence percentages are reported without reference to established thresholds for species differentiation in plants, making it difficult to assess their biological significance.
**Suggestion:** After this sentence, cite published ITS divergence thresholds (e.g., >3% for species-level differences in angiosperms) to contextualize the 12% divergence between D. binata/D. spathulata as strongly supporting their distinct species status.

**Commented [60]: Issue:** The genetic divergence percentages (88% identity, 3% indels) are presented without statistical context (e.g., p-values, confidence intervals), leaving the analytical method unclear.
**Suggestion:** Add a brief description of the statistical method used to calculate divergence (e.g., "Pairwise sequence comparisons using [method name] revealed…") in this sentence.

**Commented [61]: Issue:** This sentence provides genetic divergence values but does not contextualize how these differences support the use of ITS for species verification.
**Suggestion:** Insert a sentence before this sentence to note that divergence thresholds in ITS are established for species discrimination in plants.

Renner and Specht 2011). Their results showed that within one monophyletic *Drosera* group *D. spathulata* and *D. rotundifolia* both occurred in a *capensis* clade, and *D. binata* was grouped in a *peltata* clade.

As stated above, *D. rotundifolia* and *D. spathulata* showed high genetic relatedness, as the analysed ITS sequences were 96% identical. *Drosera rotundifolia* has round or nearly round leaf blades that are wider than they are longer, whereas *D. spathulata*, which includes diploids, tetraploids and hexaploids, can have obovate to spathulate leaf shapes. Molecular analysis appears to be justified, especially in the cases of vague morphological differences. To simplify the process of the molecular identification of *D. binata*, *D. rotundifolia* and *D. spathulata* maintained as clones under *in vitro* conditions, we chose suitable restriction enzymes conferring specific restriction profiles of ~350-bp ITS DNA sequences to corresponding taxa. As shown in Fig. 2, the restriction endonuclease *Bgl*I is applicable to the differentiation of *D. spathulata* from *D. rotundifolia* and *D. binata*, and the restriction enzyme *Sph*I conferred a specific restriction profile to *D. binata*.

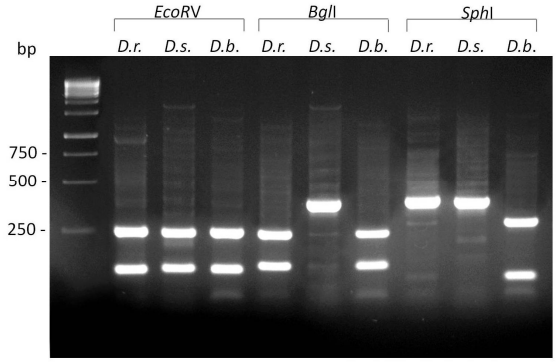



Fig. 2 Restriction analysis of the 18S ribosomal DNA (rDNA) polymerase chain reaction (PCR) fragment ~350 bp in length amplified with *Drosera rotundifolia*, *Drosera spathulata* and *Drosera binata* templates. Restriction fragments 110 bp and 240 bp long were identified in all tested samples following restriction by the *Eco*RV enzyme. Restriction analysis with *Bgl*I resulted in 135-bp and 215-bp products when the PCR products *of D. rotundifolia* and *D. binata* were digested. The restriction endonuclease *Sph*I specifically digested the PCR product of *D. binata*, and the lengths of restriction products were 100 bp and 250 bp.

The latest studies report the identification of approximately 250 species within the genus *Drosera* L. (Gonella et al. 2016). However, discoveries of new taxa (Gonella 2015) as well as the taxonomic revision of some *Drosera* species (Rivadavia et al. 2014) indicate that this

**Commented [62]: Issue:** This sentence cites previous phylogenetic studies but does not explicitly contrast their methodologies (rbcL/18S rDNA) with the current ITS-based approach, missing an opportunity to highlight the methodological advance of this work.
**Suggestion:** After this sentence, add a comparison of resolution power between ITS markers used here and the rbcL/18S markers from cited studies, noting how ITS variability better suits species-level discrimination in Drosera clones.

**Commented [63]: Issue:** This sentence compares results to prior phylogenetic studies, which is interpretive and should be reserved for the Discussion.
**Suggestion:** Move this sentence to the Discussion and replace it with a data-driven conclusion (e.g., "These divergence patterns align with known clade distinctions").

**Commented [64]: Issue:** The discussion of clade placement (capensis vs. peltata) is not explicitly linked to the study's ITS divergence results, creating a disjo ... [51]

**Commented [65]: Issue:** The clade membership discussion does not address how the current ITS d ... [52]

**Commented [66]: Issue:** The clade classifications (capensis, peltata) are described without referenc ... [53]

**Commented [67]: Issue:** This restatement of genetic relatedness between D. rotundifolia and D. spathu ... [54]

**Commented [68]: Issue:** This sentence redundantly reiterates the 96% identity between D. rotundifolia ... [55]

**Commented [69]: Issue:** The description of leaf morphology differences does not reconcile how ... [56]

**Commented [70]: Issue:** this sentence describes leaf morphology but does not explicitly link this to the s ... [57]

**Commented [71]: Issue:** This statement about morphological ambiguity justifying molecular anal ... [58]

**Commented [72]: Issue:** The rationale for using restriction enzymes is framed as a simplification b ... [59]

**Commented [73]: Issue:** The rationale for selecting restriction enzymes is described procedurally but ... [60]

**Commented [74]: Issue:** The rationale for using restriction enzymes is introduced abruptly without ... [61]

**Commented [75]: Issue:** This sentence introduces restriction profiling but does not clarify how enzym ... [62]

**Commented [76]: Issue:** The reference to Figure 2 in describing restriction profiles contradicts ... [63]

**Commented [77]: Issue:** Figure 2 is cited without first describing the key results shown in the figure (e.g., ... [64]

**Commented [78]: Issue:** This general statement about species numbers does not leverage the study's sp ... [65]

**Commented [79]: Issue:** This sentence discusses the total number of Drosera species, which is tangenti ... [66]

**Commented [80]: Issue:** This sentence cites Gonella et al. (2016) for species count but does not connect t ... [67]

number is not complete. In addition to morphological characterisation, molecular analysis appears to be a useful tool for phylogenetic studies, the identification of new taxa and the verification of individual species.

## Conclusion

Here, we verified the identity of putative *D. rotundifolia*, *D. spathulata* and *D. binata* clones cultured under *in vitro* conditions. For this, primer pairs amplifying the partial sequence of 45S rDNA covering the ITS1 5.8S and ITS2 coding sequences were used. Verification of tested putative species was performed based on the sequences obtained after both sequencing and sequence alignment with corresponding accessions present in the NCBI database. For rapid screening of these taxa, restriction analysis is also recommended.


## Acknowledgements

This work was supported by the COST FA 1208 and VEGA 2/0075/17 projects as well as by the Research Centre AgroBioTech built-in framework of European Community Project and the Building Research Centre 'AgroBioTech' ITMS 26220220180.


**Commented [81]: Issue:** The statement about molecular analysis being "useful" is vague and does not emphasize how this study's approach addresses specific gaps in Drosera taxonomy or conservation.
**Suggestion:** After this sentence, add a sentence specifying that the combined ITS sequencing and restriction profiling method enables precise clone verification for conservation programs aiming to preserve genetic integrity in ex situ collections.

**Commented [82]: Issue:** The concluding statement about molecular analysis being "useful" is underdeveloped compared to recommendations to specify concrete applications, particularly given the study's demonstration of restriction profiling for clone verification.
**Suggestion:** After this sentence, explicitly propose three applications: 1) quality control in botanical collections, 2) validation of hybrid specimens, and 3) screening of field-collected material during taxonomic surveys.

**Commented [83]: Issue:** This sentence advocates for molecular analysis as a taxonomic tool, which is an interpretive conclusion better suited to the Discussion.
**Suggestion:** Relocate this sentence to the Discuss... [68]

**Commented [84]: Issue:** The Conclusion fails to connect the work to real-world applications, such... [69]

**Commented [85]: Issue:** The Conclusion section lacks a forward-looking perspective, such as testable... [70]

**Commented [86]: Issue:** The Conclusion does not contextualize the study within the ongoing taxonor... [71]

**Commented [87]: Issue:** The Conclusion does not address the implications of the observed genetic... [72]

**Commented [88]: Issue:** This sentence restates the primary finding of the study without situating it wit... [73]

**Commented [89]: Issue:** The Conclusion section only restates methodological steps without synthesizir... [74]

**Commented [90]: Issue:** The conclusion in this sentence restates the verification of clones but do... [75]

**Commented [91]: Issue:** This sentence reiterates methodological details already described in the Re... [76]

**Commented [92]: Issue:** This sentence does not address the limitations of relying on NCBI accessio... [77]

**Commented [93]: Issue:** This sentence summarizes the verification process without explaining how this w... [78]

**Commented [94]: Issue:** The conclusion recommends restriction analysis but does not address limitation... [79]

**Commented [95]: Issue:** The recommendation for restriction analysis is presented without acknowle... [80]

**Commented [96]: Issue:** This sentence recommends restriction analysis but does not clarify its practica... [81]

**Commented [97]: Issue:** The final recommendation for restriction analysis is disconnected from practical... [82]

**Commented [98]: Issue:** The final recommendation in this sentence is vague and does not prioritize spec... [83]

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**Issue:** The title includes "Drosera binata var. Dichotoma," but the Introduction and subsequent sections refer only to "Drosera binata" without specifying the variety, creating inconsistency. This omission may confuse readers about the exact taxonomic scope of the study.

**Suggestion:** Clarify whether the study includes the Dichotoma variety or focuses solely on the broader D. binata species. If the variety is relevant, ensure its mention is consistent throughout the manuscript.

**Page 1: [2] Commented [3]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The title does not clearly state the main conclusion or novel aspect of the study, such as the development of restriction profiles for differentiation, which limits its ability to convey the specific contribution.

**Suggestion:** Revise the title to emphasize the novel method (e.g., restriction profiling) and its utility for distinguishing species. Example: *"Differentiation of in vitro-grown Drosera clones using ITS sequencing and restriction profiling confirms morphological ambiguity in carnivorous plants."*

**Page 1: [3] Commented [4]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The abstract's opening sentence mentions the incomplete species count but does not tie this to the central problem of taxonomic uncertainty in cultivated clones, missing an opportunity to frame the study's significance.

**Suggestion:** After this sentence, add a sentence explaining that taxonomic ambiguities are exacerbated in vitro, where morphological traits become unreliable, necessitating molecular verification.

**Page 1: [4] Commented [5]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence begins the abstract with general background about Drosera species diversity but does not establish the specific motivation or knowledge gap (e.g., challenges in verifying clones due to morphological plasticity under in vitro conditions).

**Suggestion:** Add a sentence after this sentence to clarify the problem: *"Morphological traits used for species identification, such as leaf shape, are often unstable in vitro, complicating clone verification."*

**Page 1: [5] Commented [6]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence introduces the study's focus but does not explicitly connect the need for molecular verification to the broader challenge of morphological plasticity in vitro, which is critical for justifying the work's novelty.

**Suggestion:** After this sentence, add a sentence explaining how in vitro propagation can obscure morphological traits used in traditional taxonomy, necessitating molecular methods for reliable clone identification.

**Page 1: [6] Commented [7]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The aim statement in this sentence is overly technical and does not explicitly frame the study's purpose (e.g., resolving taxonomic uncertainty in clones).

**Suggestion:** Rephrase the aim to highlight the practical goal. Add after this sentence: *"This study aimed to resolve taxonomic uncertainty in in vitro clones by combining ITS sequencing with restriction profiling."*

**Page 1: [7] Commented [8]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The statement about restriction profiles enabling differentiation of D. rotundifolia and D. spathulata lacks a clear link to practical applications, such as how this method improves efficiency over existing techniques.

**Suggestion:** After this sentence, add a sentence clarifying that these restriction profiles provide a cost-effective, rapid alternative to full sequencing for routine clone verification in botanical collections or conservation programs.

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**Issue:** The abstract's results section lacks quantitative data (e.g., sequence identity percentages or restriction fragment sizes) to support claims of differentiation between species.

**Suggestion:** Insert specific values from the results (e.g., 96% identity between D. rotundifolia and D. spathulata) after this sentence to strengthen the conclusion.

**Page 1: [9] Commented [10]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The keywords omit critical terms like "restriction profiling" and "clonal verification," reducing discoverability.

**Suggestion:** Revise keywords to include "restriction enzyme analysis" and "in vitro clone identification" in this sentence.

**Page 1: [10] Commented [11]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence lists multiple citations for the utility of ITS and rbcL markers but does not explicitly state why ITS was prioritized over rbcL or other markers for this study. The rationale for selecting ITS as the primary tool for clone verification is unclear, weakening the logical flow.

**Suggestion:** After this sentence, add a brief explanation of why ITS is particularly suited for resolving intraspecific variation in Drosera clones, especially in the context of in vitro morphological plasticity.

**Page 1: [11] Commented [12]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The discussion of rbcL analysis in Droseraceae phylogeny is overly detailed and tangential to the study's focus on verifying clones using ITS markers. This distracts from the central narrative about molecular identification challenges in cultivated Drosera.

**Suggestion:** Condense or remove this sentence to focus on ITS-specific background. If retained, add a transition after this sentence linking rbcL's limitations to ITS's advantages for clonal verification.

**Page 2: [12] Commented [14]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The discussion of fly traps and glandular hairs is irrelevant to the study's focus on molecular verification. This deviation disrupts the logical flow and fails to advance the rationale for using ITS markers.

**Suggestion:** Remove or replace this sentence with a discussion of morphological traits (e.g., leaf shape) that are unreliable for taxonomy in vitro, directly tying to the need for molecular methods.

**Page 2: [13] Commented [15]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence describes leaf shape as a "morphologically distinct trait," which contradicts the Abstract's assertion that leaf shape is not a reliable diagnostic feature. This inconsistency undermines the study's motivation.

**Suggestion:** Revise this sentence to emphasize that leaf shape, while historically used, is insufficient for taxonomy in vitro, necessitating molecular tools. Place this after this sentence to strengthen the rationale.

**Page 2: [14] Commented [16]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The mention of RAPD and ITS markers does not clarify why ITS was chosen over RAPD for this study. The absence of a comparative rationale leaves readers questioning the methodological

choice.

**Suggestion:** After this sentence, add a sentence explaining why ITS offers higher resolution or reproducibility compared to RAPD for verifying in vitro clones, citing relevant literature if available.

**Page 2: [15] Commented [17]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence states that ITS divergence resolves species within genera but does not address its applicability to clonal verification, which is the study's focus. The link between ITS utility and the specific challenge of in vitro clones is missing.

**Suggestion:** After this sentence, add a statement connecting ITS's resolution power to the need for accurate identification of clones whose morphology may be altered by in vitro conditions.

**Page 2: [16] Commented [18]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The sentence states the use of ITS markers but does not contextualize why ITS was selected over other markers (e.g., rbcL) for this specific application, weakening the methodological rationale.

**Suggestion:** Before this sentence, add a sentence noting that ITS was chosen due to its higher divergence compared to chloroplast markers, making it more suitable for resolving closely related Drosera species.

**Page 2: [17] Commented [19]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The aim statement lacks a clear articulation of the knowledge gap—specifically, why verifying in vitro clones of these species is novel or necessary. This weakens the "hook" required to engage readers.

**Suggestion:** Before this sentence, add a sentence highlighting the lack of standardized molecular methods for verifying Drosera clones in vitro, particularly for species with overlapping morphologies.

**Page 2: [18] Commented [20]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence states the use of ITS markers but does not justify why ITS was selected over other markers (e.g., rbcL) for this specific application, weakening the rationale.

**Suggestion:** Add a sentence before this sentence to explain the advantages of ITS for intragenus differentiation, referencing its higher mutation rate compared to coding regions.

**Page 2: [19] Commented [21]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The mention of "proposed a specific restriction profile" is abrupt and lacks justification for why restriction analysis was prioritized alongside sequencing.

**Suggestion:** After this sentence, add a sentence explaining that restriction profiling was developed to complement sequencing, enabling laboratories without sequencing capabilities to perform reliable identifications.

**Page 2: [20] Commented [22]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The final sentence of the Introduction mentions restriction profiles but does not emphasize the broader significance of the findings, such as their practical utility for laboratories or conservation efforts.

**Suggestion:** After this sentence, add a concluding statement underscoring how the proposed restriction profiles streamline clone verification, reducing reliance on sequencing in resource-limited settings.

**Page 2: [21] Commented [23]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The origin of plant material is described as "provided as gifts," which lacks critical details such as the number of clones analyzed, their geographic origins, or voucher specimen deposition, raising reproducibility concerns.

**Suggestion:** In this sentence, specify the number of clones per species, their source populations, and whether voucher specimens were deposited in a public herbarium.

**Page 2: [22] Commented [24]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The origin of plant material is described as a gift, but there is no mention of voucher specimen deposition in a herbarium or seed bank, which is standard practice for taxonomic verification.

**Suggestion:** After this sentence, state whether voucher specimens were deposited in a recognized institution and provide accession numbers to confirm taxonomic identity.

**Page 2: [23] Commented [25]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence cites cultivation methods from a previous study but does not provide essential details about the in vitro growth conditions (e.g., media composition, light/dark cycles, temperature, or duration of cultivation), which are critical for replication.

**Suggestion:** After this sentence, add specifics such as the agar concentration, nutrient

components (e.g., Murashige and Skoog basal salts), pH, photoperiod, temperature regime, and duration of cultivation to ensure reproducibility.

**Page 2: [24] Commented [26]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** this sentence does not specify the number of clones analyzed per species or replication details, raising concerns about experimental rigor.

**Suggestion:** Clarify the sample size (e.g., *"Three clones per species were analyzed"*) in this sentence to strengthen reproducibility.

**Page 2: [25] Commented [27]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence references a DNA isolation protocol but lacks technical details (e.g., whether a commercial kit or CTAB-based method was used, buffer compositions, or centrifugation parameters), which limits reproducibility.

**Suggestion:** After this sentence, clarify the DNA extraction method by specifying the protocol steps (e.g., lysis buffer components, RNase treatment, precipitation steps) or cite a more detailed protocol if applicable.

**Page 3: [26] Commented [30]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence describes primer design but does not justify why these specific primers were selected over alternatives or how they were validated for specificity across the three Drosera species, which is critical for methodological rigor.

**Suggestion:** After this sentence, include a brief rationale for primer selection (e.g., alignment of conserved regions across species) and mention any in silico validation steps (e.g., BLAST analysis) to confirm specificity.

**Page 3: [27] Commented [31]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence describes primer design but does not address potential cross-species amplification risks, which is critical for specificity.

**Suggestion:** Add a sentence after this sentence to note that primers were validated against non-target species to ensure specificity.

**Page 3: [28] Commented [32]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The PCR conditions omit critical details such as the type of thermocycler used, reaction volume, and concentrations of reagents (e.g., $MgCl_2$, dNTPs, polymerase), which are necessary for

replication.

**Suggestion:** After this sentence, specify the thermocycler model, reaction volume, and reagent concentrations (e.g., 1.5 mM $MgCl_2$, 0.2 mM dNTPs) to ensure experimental reproducibility.

**Page 3: [29] Commented [33]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The sequencing methodology does not specify the number of replicates per sample or how sequence discrepancies were resolved (e.g., consensus generation from multiple clones), raising concerns about data reliability.

**Suggestion:** After this sentence, state the number of biological replicates and the approach for resolving sequence ambiguities (e.g., majority rule or manual inspection).

**Page 3: [30] Commented [34]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence states that PCR products were cloned and sequenced but does not specify the number of clones sequenced per species, the criteria for selecting clones, or whether bidirectional sequencing was performed, raising concerns about data robustness.

**Suggestion:** After this sentence, clarify the number of clones sequenced per species, the selection process (e.g., random picking), and confirm bidirectional sequencing to validate accuracy.

**Page 3: [31] Commented [35]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence lists NCBI accession numbers for reference sequences but does not state whether the newly generated sequences from this study were deposited in a public database, which is a requirement for transparency and data accessibility.

**Suggestion:** After this sentence, add a statement confirming deposition of the newly obtained sequences in NCBI GenBank or another repository, including accession numbers if available.

**Page 3: [32] Commented [36]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The alignment software (CLUSTAL O) is cited, but the parameters used (e.g., gap penalties, substitution matrix) are not provided, which is inconsistent with recommendations to ensure computational reproducibility.

**Suggestion:** After this sentence, include alignment parameters (e.g., default settings or custom gap opening/extension penalties) to enable replication of bioinformatic analyses.

**Page 3: [33] Commented [37]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The use of NEBcutter v2.0 for restriction analysis is mentioned, but the parameters or settings used in the software (e.g., enzyme selection filters, buffer conditions) are not described, hindering replication.

**Suggestion:** After this sentence, specify the software settings (e.g., default parameters, enzyme selection criteria) and whether in silico predictions were experimentally validated.

**Page 3: [34] Commented [38]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The use of EcoRI, BglI, and SphI is stated without justification for their selection, leaving readers uncertain whether these enzymes were empirically validated or chosen in silico.

**Suggestion:** Before this sentence, add a sentence explaining that enzyme selection was based on in silico analysis of ITS sequences to target species-specific restriction sites absent in close relatives.

**Page 3: [35] Commented [39]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Materials and Methods section mentions EcoRI use without later reporting its results, creating expectation for unused data that violates recommendations against discussing unshown data.

**Suggestion:** Either remove EcoRI from this sentence or add a brief explanation in the Results and Discussion (after this sentence) noting that EcoRI produced non-diagnostic patterns and was therefore excluded from final analyses.

**Page 3: [36] Commented [40]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence names restriction enzymes but omits digestion conditions (e.g., incubation time, temperature, buffer composition) and how fragment sizes were determined (e.g., gel electrophoresis details), which are essential for reproducibility.

**Suggestion:** After this sentence, describe the restriction digest protocol (e.g., 37°C for 1 hour in NEBuffer 3.1) and electrophoresis parameters (e.g., agarose percentage, voltage, DNA ladder used).

**Page 3: [37] Commented [41]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence mentions restriction enzymes but does not report validation of digest results (e.g., gel electrophoresis images in supplements).

**Suggestion:** Include a reference to supplementary data (e.g., *"Digest results were confirmed by agarose gel electrophoresis (Supplementary Fig. S1)"*) in this sentence.

**Page 3: [38] Commented [42]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This section combines Results and Discussion, which is inconsistent with recommendations to separate these sections to allow focused interpretation of findings in the context of existing literature. The interleaving of results reporting with discussion elements reduces clarity.

**Suggestion:** Restructure the manuscript to create distinct Results and Discussion sections. Move all interpretive content (e.g., comparisons to previous studies in sentences 49-50, implications in this sentence) to a dedicated Discussion section. Place this new Discussion section after the Results.

**Page 3: [39] Commented [43]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Results section lacks subheadings, which are recommended to improve readability and highlight the logical progression of experiments.

**Suggestion:** Add subheadings (e.g., “ITS Sequence Analysis” before this sentence; “Restriction Profile Differentiation” before this sentence) to structure the content.

**Page 3: [40] Commented [44]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Results and Discussion sections are combined, which is inconsistent with recommendations to present results objectively before interpreting their significance in a separate Discussion section. This merging reduces clarity and disrupts the logical flow from data presentation to interpretation.

**Suggestion:** Separate the Results and Discussion sections. Move all interpretive content (e.g., comparisons to prior studies, broader implications) to a dedicated Discussion section. For example, sentences 49, 50, 60, and 62 should be relocated.

**Page 3: [41] Commented [45]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence begins the Discussion by emphasizing verification needs without first summarizing key findings, which is inconsistent with recommendations to open Discussions with a concise synthesis of study aims and principal outcomes.

**Suggestion:** Add an opening paragraph before this sentence that explicitly states: 1) the study's rationale for using ITS markers in clone verification, 2) the key technical achievement (successful differentiation via ITS sequencing/RFLP), and 3) the broader implication for maintaining genetic fidelity in in vitro cultures.

**Page 3: [42] Commented [46]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence cites previous studies (Tungkajiwangkoon et al. 2016; Batagin et al. 2009; Trejgell et al. 2012), which is inconsistent with recommendations to avoid referencing other works in the Results section. Such comparisons belong in the Discussion.

**Suggestion:** Remove citations to prior studies here. Relocate this contextual discussion to the Introduction or Discussion.

**Page 3: [43] Commented [47]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The reference to future gene expression studies is tangential to the manuscript's focus on molecular verification and distracts from the core findings.

**Suggestion:** Remove the phrase "precedes the isolation and expression profile characterisation of other genes" from this sentence to maintain focus on the current work's objectives.

**Page 3: [44] Commented [48]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The mention of future gene expression studies introduces an unrelated research direction without justifying its relevance to the current work on clone verification, creating a conceptual diversion.

**Suggestion:** After this sentence, add a transition explaining how accurate species identification is prerequisite for comparative gene studies, thereby establishing the present work's foundational importance for subsequent functional genomics in Drosera.

**Page 3: [45] Commented [49]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence describes the rationale for future work ("precedes the isolation and expression profile characterisation of other genes"), which is inappropriate for the Results section. The Results should focus on presenting data, not justifying subsequent studies.

**Suggestion:** Remove this sentence or move it to the Discussion to contextualize the broader research goals.

**Page 3: [46] Commented [50]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The description of PCR fragment lengths (364 bp and 368 bp) lacks a clear connection to the experimental objective. The Results section should explicitly state the purpose of each experiment before presenting data.

**Suggestion:** Add a sentence before this sentence stating the objective (e.g., “To verify the identity of in vitro clones, we amplified ITS regions of 45S rDNA”).

**Page 3: [47] Commented [51]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence reports fragment lengths but does not explain how these sizes relate to the ability to differentiate species, leaving the significance unclear.

**Suggestion:** Add a sentence after this sentence to link fragment size consistency to reliable amplification across taxa.

**Page 3: [48] Commented [52]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence explains primer design limitations, which is methodological detail that belongs in the Methods section. Including this in Results disrupts the narrative focus on findings.

**Suggestion:** Move this explanation to the Methods section (e.g., after this sentence).

**Page 3: [49] Commented [53]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This confirmation of clone identity through NCBI alignment is presented as a procedural outcome rather than a validation of the ITS marker system's effectiveness, missing an opportunity for methodological discussion.

**Suggestion:** After this sentence, add a comparison to other molecular markers used in Drosera taxonomy (e.g., rbcL from this sentence), noting advantages of ITS for fine-scale discrimination at the species level.

**Page 3: [50] Commented [54]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The alignment results are described without referencing the corresponding figure (Fig. 1), which is inconsistent with recommendations to cite figures immediately after describing the data they represent.

**Suggestion:** Insert “(Fig. 1)” after “analysed in vitro clones” in this sentence to direct readers to the supporting figure.

**Page 5: [51] Commented [64]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The discussion of clade placement (capensis vs. peltata) is not explicitly linked to the study’s ITS divergence results, creating a disjointed narrative.

**Suggestion:** After this sentence, add a sentence connecting the observed 96% ITS identity

between D. spathulata and D. rotundifolia to their shared capensis clade, reinforcing the phylogenetic consistency of the data.

**Page 5: [52] Commented [65]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The clade membership discussion does not address how the current ITS data supports or challenges existing phylogenetic models, missing an opportunity for conceptual synthesis.
**Suggestion:** After this sentence, add analysis of whether the ITS divergence patterns align with traditional clade divisions based on morphology/biogeography, or suggest need for clade redefinition using molecular criteria.

**Page 5: [53] Commented [66]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The clade classifications (capensis, peltata) are described without referencing a figure or table that supports this result, reducing transparency.
**Suggestion:** Add a cross-reference to a figure or table (e.g., “as illustrated in Fig. 1”) in this sentence.

**Page 5: [54] Commented [67]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This restatement of genetic relatedness between D. rotundifolia and D. spathulata repeats Results content without advancing the discussion, which is inconsistent with recommendations to minimize Results repetition in Discussions.
**Suggestion:** Replace this sentence with a synthesis of how the 96% identity score impacts taxonomic delineation, considering whether this supports maintaining them as distinct species or suggests recent divergence requiring further study.

**Page 5: [55] Commented [68]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence redundantly reiterates the 96% identity between D. rotundifolia and D. spathulata, which was already stated in this sentence. Repetition disrupts conciseness.
**Suggestion:** Remove this sentence or merge it with this sentence to avoid redundancy.

**Page 5: [56] Commented [69]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The description of leaf morphology differences does not reconcile how molecular data resolves phenotypic plasticity caused by in vitro conditions, which was a key motivation stated in the Abstract.
**Suggestion:** After this sentence, explicitly state that molecular verification prevents

misidentification of clones showing atypical leaf morphologies due to culture conditions, directly addressing the problem outlined in this sentence.

**Page 5: [57] Commented [70]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** this sentence describes leaf morphology but does not explicitly link this to the study's finding that these traits are unreliable under in vitro conditions.

**Suggestion:** Add a sentence after this sentence to state that observed genetic divergence contrasts with overlapping leaf morphologies in vitro.

**Page 5: [58] Commented [71]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This statement about morphological ambiguity justifying molecular analysis is presented as a standalone observation rather than being connected to the study's specific findings about ITS variability between morphologically similar species.

**Suggestion:** In this sentence, integrate reference to the quantified genetic divergence data from this sentence (88% vs 96% identity) to directly demonstrate how molecular analysis resolves morphological uncertainties in these clones.

**Page 5: [59] Commented [72]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The rationale for using restriction enzymes is framed as a simplification but does not quantify advantages (e.g., time/cost savings) over sequencing, limiting the perceived utility.

**Suggestion:** After this sentence, add a sentence comparing the time and cost required for restriction profiling versus full sequencing, emphasizing practicality for high-throughput clone verification.

**Page 5: [60] Commented [73]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The rationale for selecting restriction enzymes is described procedurally but lacks discussion of how this approach improves upon existing identification methods, which is inconsistent with recommendations to emphasize methodological advances.

**Suggestion:** After this sentence, add a comparison to conventional ITS sequencing workflows, noting how the developed RFLP protocol reduces time/costs while maintaining accuracy for routine clone verification in laboratory settings.

**Page 5: [61] Commented [74]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The rationale for using restriction enzymes is introduced abruptly without stating the objective of this experiment, leaving the purpose unclear.

**Suggestion:** Add a sentence before this sentence explaining the goal (e.g., “To develop a rapid diagnostic tool, we identified restriction enzymes yielding species-specific profiles”).

**Page 5: [62] Commented [75]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence introduces restriction profiling but does not clarify how enzyme selection was optimized (e.g., in silico analysis vs. empirical testing).

**Suggestion:** Add a sentence after this sentence to describe the criteria for enzyme selection (e.g., unique recognition sites in aligned sequences).

**Page 5: [63] Commented [76]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The reference to Figure 2 in describing restriction profiles contradicts recommendations to avoid figure citations in Discussions, while also repeating Results-section details.

**Suggestion:** Remove "As shown in Fig. 2" and reframe to emphasize the practical utility of these enzyme selections rather than experimental results. Place this revised content in this sentence instead of 55.

**Page 5: [64] Commented [77]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** Figure 2 is cited without first describing the key results shown in the figure (e.g., fragment sizes), forcing readers to consult the figure prematurely.

**Suggestion:** Describe the restriction fragment results (e.g., “BglI produced 135-bp and 215-bp fragments in D. rotundifolia and D. binata”) before citing Fig. 2 in this sentence.

**Page 5: [65] Commented [78]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This general statement about species numbers does not leverage the study's specific findings about ITS variability to comment on taxonomic challenges in Drosera, missing an opportunity to contextualize the work within ongoing taxonomic revisions.

**Suggestion:** After this sentence, add a discussion of how the developed ITS-RFLP method could aid in characterizing newly discovered taxa (per Gonella 2015) by providing a rapid screening tool before full sequencing.

**Page 5: [66] Commented [79]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence discusses the total number of Drosera species, which is tangential to the study's focus on molecular verification and belongs in the Discussion.

**Suggestion:** Move this sentence to the Discussion to contextualize the broader taxonomic challenges.

**Page 5: [67] Commented [80]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence cites Gonella et al. (2016) for species count but does not connect this to the study's relevance in improving taxonomic accuracy.

**Suggestion:** Insert a sentence after this sentence to argue that molecular tools are essential for updating Drosera taxonomy as new species are discovered.

**Page 6: [68] Commented [83]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence advocates for molecular analysis as a taxonomic tool, which is an interpretive conclusion better suited to the Discussion.

**Suggestion:** Relocate this sentence to the Discussion and replace it with a data-driven summary (e.g., "Our restriction profiles enable efficient differentiation of the studied taxa").

**Page 6: [69] Commented [84]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Conclusion fails to connect the work to real-world applications, such as how molecular verification of in vitro clones could improve the reliability of medicinal or horticultural products derived from Drosera.

**Suggestion:** After this sentence, discuss the economic or ecological implications of accurate clone identification, such as preventing mislabeling in commercial trade or ensuring genetic fidelity in reintroduction programs for threatened Drosera species.

**Page 6: [70] Commented [85]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Conclusion section lacks a forward-looking perspective, such as testable hypotheses or specific recommendations for future research (e.g., applying this method to newly discovered Drosera species or hybrids).

**Suggestion:** After this sentence, propose studies to validate the ITS-restriction profiling approach across a wider range of Drosera species, including polyploid complexes, and suggest integrating it with other markers (e.g., chloroplast DNA) to resolve deeper phylogenetic uncertainties.

**Page 6: [71] Commented [86]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Conclusion does not contextualize the study within the ongoing taxonomic revisions of Drosera, such as how molecular tools could resolve controversies in recently described or reclassified species.

**Suggestion:** After this sentence, highlight the role of ITS-based verification in supporting taxonomic revisions, particularly for morphologically cryptic species, and suggest applying this method to type specimens or historical herbarium samples to resolve long-standing uncertainties.

**Page 6: [72] Commented [87]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Conclusion does not address the implications of the observed genetic divergence between D. spathulata and D. binata (88% identity) versus D. spathulata and D. rotundifolia (96% identity) for Drosera phylogenetics or taxonomy.

**Suggestion:** After this sentence, add a brief discussion of how these divergence levels align with existing clade classifications (capensis vs. peltata) and whether they support or challenge current taxonomic boundaries, emphasizing the need for multilocus analyses to refine species relationships.

**Page 6: [73] Commented [88]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence restates the primary finding of the study without situating it within the broader context of the field or addressing its implications for future research or applications.

**Suggestion:** After this sentence, add a discussion of how this verification method addresses challenges in carnivorous plant taxonomy, particularly for in vitro clones, and its potential to streamline conservation efforts or breeding programs. Highlight the relevance of reliable molecular identification for resolving taxonomic ambiguities in Drosera, especially given the genus's ongoing discovery of new taxa.

**Page 6: [74] Commented [89]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The Conclusion section only restates methodological steps without synthesizing the broader significance of successfully verifying in vitro clones, which is inconsistent with recommendations to emphasize scientific contributions in closing paragraphs.

**Suggestion:** Before this sentence, add a final Discussion paragraph that: 1) summarizes the validation protocol's reliability, 2) discusses implications for conservation of threatened Drosera species through verified in vitro propagation, and 3) proposes using this approach for other carnivorous plant genera.

**Page 6: [75] Commented [90]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The conclusion in this sentence restates the verification of clones but does not address broader implications, such as the utility of restriction profiling for rapid screening in plant conservation or taxonomy.

**Suggestion:** Add a sentence after this sentence to explicitly state the methodological impact: *"This approach provides a cost-effective tool for verifying Drosera clones in botanical collections and breeding programs."*

**Page 6: [76] Commented [91]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence reiterates methodological details already described in the Results and Methods sections, which is redundant and does not advance the Conclusion's purpose of synthesizing new insights.

**Suggestion:** Replace this sentence (or repurpose its placement) by inserting after this sentence a discussion of how the ITS-based approach compares to traditional morphological or other molecular markers (e.g., rbcL) for Drosera identification, emphasizing its advantages for in vitro studies where phenotypic plasticity complicates taxonomy.

**Page 6: [77] Commented [92]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence does not address the limitations of relying on NCBI accessions, which may contain errors or lack representation of intraspecific diversity, potentially affecting verification accuracy.

**Suggestion:** After this sentence, propose curating a dedicated Drosera ITS reference database with vouchered specimens to enhance reliability, and advocate for collaborative efforts among researchers to expand sequence coverage.

**Page 6: [78] Commented [93]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** This sentence summarizes the verification process without explaining how this work advances the field or addresses unresolved questions, such as the applicability of ITS markers to other Drosera species or the limitations of relying on NCBI accessions.

**Suggestion:** After this sentence, add a statement about the broader utility of this approach for validating in vitro collections in botanical gardens or research institutions, and note the need for expanded reference databases to improve accuracy, especially for understudied Drosera taxa.

**Page 6: [79] Commented [94]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The conclusion recommends restriction analysis but does not address limitations, such as potential intraspecific variability or the need for sequencing in ambiguous cases.

**Suggestion:** After this sentence, add a sentence acknowledging that restriction profiles should be validated against sequencing data for novel variants or populations with unknown genetic diversity.

**Page 6: [80] Commented [95]** **Scientific-editing.ai** **3/7/2026 10:17:00 PM**

**Issue:** The recommendation for restriction analysis is presented without acknowledging potential pitfalls, such as intra-specific variation in restriction sites or the risk of misidentification if profiles overlap between species.

**Suggestion:** After this sentence, include a caveat about validating restriction profiles across diverse populations and recommend coupling this method with sequencing for critical identifications, ensuring robustness in practical applications.

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**Issue:** This sentence recommends restriction analysis but does not clarify its practical advantages (e.g., cost, time) over sequencing or its limitations (e.g., dependency on specific restriction sites, potential for homoplasy).

**Suggestion:** After this sentence, elaborate on scenarios where restriction profiling would be most impactful (e.g., high-throughput screening of clones in commercial nurseries) and propose validations needed before adopting this method for critical applications like species conservation or reintroduction programs.

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**Issue:** The final recommendation for restriction analysis is disconnected from practical implementation considerations, such as cost-benefit comparisons between sequencing and RFLP or equipment requirements for plant laboratories.

**Suggestion:** After this sentence, add a brief cost/accessibility analysis noting that restriction profiling requires only basic PCR infrastructure compared to sequencing, making it suitable for resource-limited laboratories maintaining in vitro collections.

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**Issue:** The final recommendation in this sentence is vague and does not prioritize specific enzymes (e.g., BglI for D. spathulata) for practical use.

**Suggestion:** Modify this sentence to specify the most effective enzymes: *"For rapid screening, BglI and SphI are recommended for differentiating D. spathulata and D. binata, respectively."*