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Distinguishing two morphologically similar species of Asteraceae using a chloroplast DNA marker

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Abstract. *Synedrella nodiflora* (L.) Gaertn and *Calypocarpus vialis* Less are members of Asteraceae family that morphologically show high similarities. To genetically distinguish between them, a particular molecular marker should be employed. This study aims to present molecular comparison between both species using a chloroplast DNA marker, i.e. *atpB* – *rbcL* IGS. A pair of PCR universal primers was used to amplify the marker. Sequence alignment on the PCR products reveals longer *S. nodiflora* sequence in comparison to that of *C. vialis*. In addition, some transversions and transitions are also observed. This suggests that the two species exhibit considerable genetic difference despite their similar phenotypic appearance.

1. Introduction

Many members of Asteraceae family are recognized for their potentials as ornamental, medicinal, and economic plants[1]. On the other hand, some others are known as invasive weeds[2], resulting in significant loss on several crops with respect to productivity[3].

Some species of Asteraceae family show very high phenotypical similarities causing difficulty in differentiating them from each other. For example, *Calypocarpus vialis* Less has ever been identified as *Synedrella vialis* (Less.) A. Gray due to its high resemblance to *Synedrella nodiflora*[4]. Nevertheless, *S. vialis* is now changed into *C. vialis* [5] and this is the scientifically accepted name for the species, while *S. nodiflora* has taxonomically been the only species of genus *Synedrella* [6].

Relatively many studies on the potentials of *S. nodiflora*, e.g. as medicinal herbs[7],[8],[9],[10],[11], bioinsecticide[12], biofungicide[13], and detoxificant for heavy metals such as Cu and Pb[14], have been reported. On the other hand, no study has been performed on *C. vialis* potentials to human life. However, this plant species is often called as straggle daisy because of its capacity to grow invasively in various terrestrial habitats[15]. The allelopathy effect of root and leaf extracts of *C. vialis* was reported to strongly inhibit *S. Nodiflora*[16],[17].

Despite its wide distribution over many tropical countries, *S. nodiflora* showed no genetic difference among various altitudes[18], while low genetic difference within *S. nodiflora* populations in Java Island, Indonesia was observed[19]. On the other hand, *C. vialis* is not only spread over tropical regions, but is also distributed throughout subtropical areas as it is native to Mexico or even Texas[20]. It seems likely that *C. vialis* is a self-pollinated species presumably leading to slightly floral morphological differences between the populations in Texas and those in Mexico, especially concerning anther number and corolla lobe number of disk florets[21]. Yet, these phenotypical variations are not sufficiently easy to see unless considerably careful examination is made. Even the difficulty occurs in the case of distinguishing *C. vialis* and *S. nodiflora*.

The problem with phenotypical discrimination between both species is necessarily overcome by means of molecular comparison using particular genetic markers, some of which are those from chloroplast genome (cpDNA). This source of molecular markers is maternally inherited in agiosperms giving rise to the absence of genetic recombination. Hence, it can be used properly for assessing both intra-specific and inter-specific genetic diversity[22]. An *atpB* – *rbcL* intergenic spacer (IGS) is one of cpDNA markers commonly used to analyze evolutionary history at lower level, since it is a non coding sequence showing high evolution rate[23],[24],[25]. This marker has been used to study population genetic structure of some Chinese endemic plant species revealing high connectivity among populations[26]. Here we present our study on the genetic comparison between *S. nodiflora* and *C. vialis* by the use of *atpB* – *rbcL* IGS as the molecular marker. It is expected from this study to obtain DNA barcoding for the respective species.

2. Materials and Methods

2.1. Plant sampling and preparation

The samples of both *S. nodiflora* and *C. vialis* were collected randomly from some sites in Banyumas Regency, Central Java, Indonesia in May 2020. Five plant individuals were used as samples of the respective species, each of which was taken by removing its roots and put the whole plant into a plastic bottle formerly filled with some water. This was then grown in the glass house of the Faculty of Biology Universitas Jenderal Soedirman. Molecular analysis was performed in the Laboratory of Genetics and Plant Breeding of the Faculty of Agriculture Universitas Gadjah Mada.

2.2. Genomic DNA extraction and marker amplification

Genomic DNAs were extracted from the uppermost leaves of the plant samples using CTAB method [27]. The extracted DNAs were then used as PCR templates to amplify *atpB* – *rbcL* IGS employing a pair of universal primers, i.e. 5' – ACATCKARTACKGGACCAATAA - 3' as forward primer and 5' - AACACCAGCTTTTRAATCCAA - 3' as reverse primer [28]. Individual PCR reaction was performed in a total volume of 10 µl consisting of 2.5 µl genomic DNA; 0.25 µl primers (0.125 µl each primer); 5 µl Gotaq green and 2.25 µl NFW. This reaction mixture was then treated in a PCR condition as follows: pre-denaturation at 94°C for 3 mins, 33 reaction cycles consisting of denaturation at 94°C for 45 secs, primer annealing at 55°C for 45 secs, extension at 72°C for 2 mins respectively, followed by final extension at 72°C for 3 mins and storage at 4°C. Visualization of the PCR products was performed in a 1.5 % agarose gel electrophoresis using 1X TAE buffer run at 75 Volt, 400 mA for 40 mins. After ethidium bromide staining, the gel was exposed to UV transiluminator for documentation.

2.3. DNA sequencing and data analysis

The PCR products were purified using QIAquick kit (Qiagen, Germany), and were sequenced following automated Sanger et al.[29] with terminator labelling. Data on base sequences were edited using Bioedit version 7.0.4.1[30] and were checked manually. Sequence alignment was carried out using ClustalW [31], which was also implemented in the Bioedit version 7.0.4.1.

3. Results and Discussion

All DNA samples were successfully amplified resulting in PCR bands of approximately 800 bp in length as depicted in Figure 1. After manual editing the amplicon sequences were trimmed into only 773 bp long. Blasting to NCBI reveals that those of *S. nodiflora* samples show 99.74% to 99.87% homology with *atpB* – *rbcL* IGS sequences of *S. nodiflora* available in the data base. Meanwhile, somewhat lower percentage of homology, i.e. 95.6% to 95.73%, was observed between amplicon sequences of *C. vialis* samples and *atpB-rbcL* IGS in the NCBI genbank (Table 1). This confirms that the PCR products of both *S. nodiflora* and *C. vialis* samples are definitely *atpB* – *rbcL* IGS.

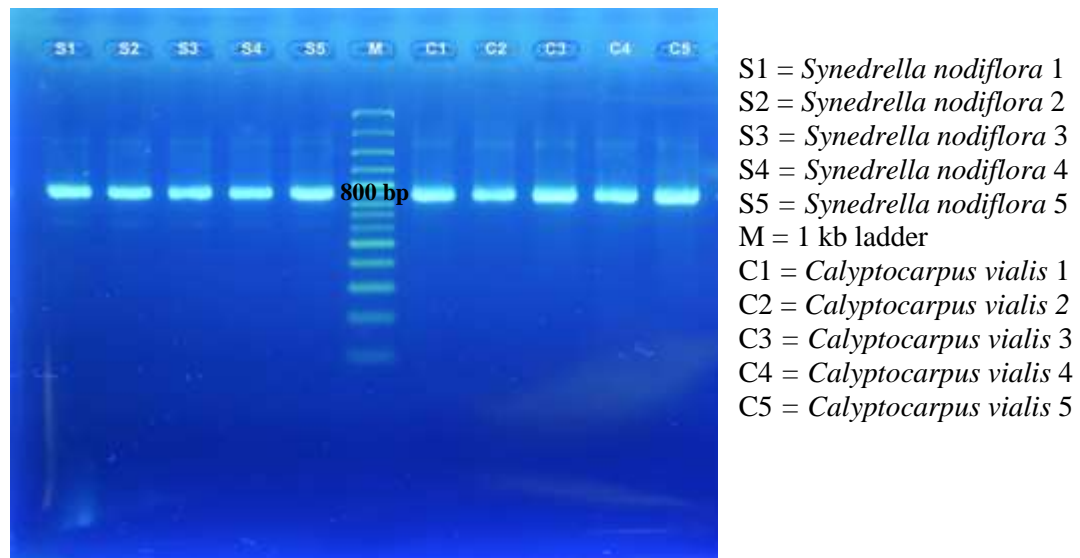


Figure 1. Amplicons of *atpB – rbcL* IGS *Synedrella nodiflora* (L.) Gaertn and *Calypotocarpus vialis* Less

Table 1. Sequence alignment of *atpB – rbcL* IGS of *Synedrella nodiflora* (L.) Gaertn and *Calypotocarpus vialis* Less to NCBI data base

No.	Sequence name	Accession number	% homology		Sequence length (bp)
			Sn	Cv	
1	<i>Synedrella nodiflora</i> haplotype 5 <i>rbcL-atpB</i>	KY983545.1	99.87	95.73	860
2	<i>Synedrella nodiflora</i> haplotype 3 <i>rbcL-atpB</i>	KY983543.1	99.87	95.73	860
3	<i>Synedrella nodiflora</i> biovar lumajang <i>rbcL-atpB</i>	KX096802.1	99.87	95.73	866
4	<i>Synedrella nodiflora</i> biovar yogya1 <i>rbcL-atpB</i>	KX096801.1	99.87	95.73	866
5	<i>Synedrella nodiflora</i> haplotype 4 <i>rbcL-atpB</i>	KY983544.1	99.74	95.60	860

Sn = *Synedrella nodiflora* (L.) Gaertn

Cv = *Calypotocarpus vialis* Less

No difference within *atpB – rbcL* IGS sequences of either *S. nodiflora* or *C. vialis* was found. On the other hand, as shown in Table 2 slightly shorter *atpB – rbcL* IGS sequence of *C. vialis* in comparison to that of *S. nodiflora* was observed due to several deletions. In addition, some base substitutions were also found, where transversion occurs more frequently rather than transition. Both *S. nodiflora* and *C. vialis atpB – rbcL* IGS sequences have now been submitted to NCBI data base for accession numbers.

Table 2. Sequence differences of *atpB – rbcL* IGS between *Synedrella nodiflora* (L.) Gaertn and *Calypocarpus vialis* Less

No.	Species	Site (s)	Sequence (s)	Type of mutation
1	<i>Synedrella nodiflora</i>	229	T	transversion
	<i>Calypocarpus vialis</i>	229	G	
2	<i>Synedrella nodiflora</i>	230	T	deletion
	<i>Calypocarpus vialis</i>	230	-	
3	<i>Synedrella nodiflora</i>	406 – 412	ATAGAAA	deletion
	<i>Calypocarpus vialis</i>	405 – 406	-	
4	<i>Synedrella nodiflora</i>	523	C	transversion
	<i>Calypocarpus vialis</i>	515	A	
5	<i>Synedrella nodiflora</i>	609 – 629	TGAAAACATTGAAATAAATAT	deletion
	<i>Calypocarpus vialis</i>	601 – 602	-	
6	<i>Synedrella nodiflora</i>	646	A	transition
	<i>Calypocarpus vialis</i>	617	T	
7	<i>Synedrella nodiflora</i>	661	G	transversion
	<i>Calypocarpus vialis</i>	632	T	
8	<i>Synedrella nodiflora</i>	683	G	transition
	<i>Calypocarpus vialis</i>	653	A	

The cpDNA marker *atpB – rbcL* has also been used previously to distinguish between *S. nodiflora* and another species of Asteraceae, i.e. *Eleutheranthera ruderalis*. These two species are also phenotypically very identical to each other. Nevertheless, by using the molecular marker some genetic differences with respect to indels and base substitutions were observed. Overall, the *atpB – rbcL* IGS of *S. nodiflora* was proven somewhat longer than that of *E. ruderalis* [32]. Oppositely, when another cpDNA marker, i.e. *trnT – trnL*, was employed to discriminate between both species, the sequence of *S. nodiflora* was found slightly shorter in comparison to that of *E. ruderalis* [33].

The *atpB – rbcL* IGS is a non-coding sequence, which is not responsible for a protein synthesis. Hence, it has no any relationship with the existence of some morphological characters observed in the plant individuals. Nevertheless, the difference in *atpB – rbcL* IGS sequences between *S. nodiflora* and *C. vialis* can potentially be used as DNA barcoding of the respective species. An intergenic spacer from cpDNA, i.e. *psbA – trnH* was used to distinguish several species of *Tolpis* (Asteraceae)[34], while the same cpDNA marker was used to provide an empirical model in the identification of some medicinal plant species of *Sinosenecio* (Asteraceae)[35]. In addition, this cpDNA marker was also used to construct phylogenetic tree among some species of *Anacyclus* (Asteraceae)[36].

Two morphologically similar genera of Myrtaceae, i.e. *Eugenia* and *Syzygium*, have been distinguished genetically employing *atpB – rbcL* IGS. By using this molecular marker, a previously confusing species, i.e. *Eugenia boerlagei* Merr, has now been taxonomically grouped into *Syzygium* rather than *Eugenia* leading to renaming this species into *Syzygium boerlagei*. However, this replacement is not based on the size of *atpB – rbcL* IGS, but rather depending on the GC content of the marker [37].

4. Conclusion

Despite no direct relationship between *atpB – rbcL* IGS and the phenotypic characters of both *S. nodiflora* and *C. vialis*, genetic differences between them were clearly observed. This provides potential DNA barcodes for identification of the two species.

References

- [1] Gao T, Yao H, Song J, Zhu Y, Liu C and Chen S. 2010. Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family. *BMC Evol. Biol.* 10: 1–7.
- [2] Souza Filho PRM. and Takaki M. 2011. Dimorphic cypsela germination and plant growth in *Synedrella nodiflora* (L.) Gaertn. (Asteraceae). *Brazilian J. Biol.* 71: 541–548.
- [3] Srithi K, Balslev H, Tanming W and Trisonthi C. 2017. Weed diversity and uses: a case study from tea plantations in northern Thailand. *Econ. Bot.* 71: 147–159.
- [4] Lal B, Prakash O, Sharma V, Singh RD and Uniyal SK. 2009. *Synedrella vialis* (Less.) A. Gray – a new record to the Flora of Himachal Pradesh. <http://www.researchgate.net/publication/236232541>.
- [5] Swetha B and Ravi Prasad B. 2013. *Calyptocarpus vialis* Less. (Asteraceae), a new distributional record for Andhra Pradesh. *J. Biosci. Res.* 14(1): 10 – 11.
- [6] The Plant List. 2013. Version 1.1. <http://www.theplantlist.org/>.
- [7] Amoteng P, Adjei S, Osei-Safo D, Kukuia KKE, Bekoe EO, Karikari TK and Kombian SB. 2017a. Extract of *Synedrella nodiflora* (L.) Gaertn exhibits antipsychotic properties in murin models of psychosis. *BMC Complementary and Alternative Med.* 17: 1 – 14.
- [8] Amoteng P, Adjei S, Osei-Safo D, Kukuia KKE, Kretchy IA, Sarkodie JA and N'guessan BB. 2017b. Analgesic effects of a hydro-ethanolic whole plant extract of *Synedrella nodiflora* (L.) Gaertn in paclitaxel-induced neuropathic pain in rats. *BMC Res. Notes* 10: 1–7.
- [9] Sekar VD, Aishwarya R, Gayathri P, Chamundeeswari D and Sangeetha M. 2018. Evaluation of antiarthritic activity of *Synedrella nodiflora* plant extracts. *Int. J. Green Pharm.* 12: 25–28.
- [10] Dhanasekar W, Aishwarya R, Chamundeeswari D and Sangeetha M. 2020. Free radical scavenging activity of the plant extract of *Synedrella nodiflora*. *Int. J. Green Pharm.* 14(3): 235 – 238.
- [11] Le HTT, Park JY, Ha J, Kusumaningrum S, Paik JH and Cho S. 2020. *Synedrella nodiflora* (Linn.) Gaertn. inhibits inflammatory responses through the regulation of Syk in RAW 264.7 macrophages. *Exp. Therapeutic Med.* 20: 1153 – 1162.
- [12] Rathi MJ and Gopalakrishnan S. 2006. Insecticidal activity of aerial parts of *Synedrella nodiflora* Gaertn (Compositae) on *Spodoptera litura* (Fab.). *J. Central European Agric.* 7(2): 289 – 296.
- [13] Sanit S. 2016. Antifungal activity of selected medicinal plants against *Alternaria* species: the pathogen of dirty panicle disease in rice. *J. Med. Plants Res.* 10(15): 195 – 201.
- [14] Prekeyi TF and Oghenekevwe O. 2007. Effects of dietary supplementation of node weed (*Synedrella nodiflora*) on toxicity of copper and lead in guinea pigs (*Cavia porcellus*). *Toxicol. Environ. Chem.* 89(2): 215 – 222.
- [15] Prasad KS and Raveendran K. 2013. *Calyptocarpus vialis* Less (Asteraceae) - a new record for Kerala, India. *Zoo's Print XXVIII*: 23 – 24.
- [16] Sagar K. 2016. Quantitative estimation of total phenols in *Calyptocarpus valis* - an emerging weed in Karnataka. *Indian J. Weed Sci.* 48(4): 470 – 472.
- [17] Sagar K. 2017. Allelopathic effect of straggler daisy (an emerging aggressive invasive weed) on its associated flora. *World J. Pharm. Res.* 7(1): 532 – 544.
- [18] Susanto AH and Dwiati M. 2020a. Molecular profile of *Synedrella nodiflora* (L.) Gaertn. from three different altitudes based on *atpB – rbcL* IGS. IOP Conference Series: Earth and Environmental Science 550, Proc. Int. Conf. Mangroves and Its Related Ecosystems 2019, Purwokerto, Indonesia, 1 – 4.
- [19] Susanto AH, Nuryanto A and Daryono BS. 2018. High connectivity among *Synedrella nodiflora* populations in Java Island based on intergeneric spacer *atpB – rbcL*. *Biosaintifika: J. Biol. Biol. Edu.* 10(1): 41 – 47.
- [20] Nesom GL. 2011. Is *Calyptocarpus vialis* (Asteraceae) native or introduced in Texas? *Phytoneuron* 1: 1 – 7.

- [21] Estes JR. 2018. Anther number, anther apical appendage, and pollination biology of *Calyptocarpus vialis* Lessing (Heliantheae: Asteraceae). *Oklahoma Native Plant Record* 18: 45 – 51.
- [22] Caron H, Molino JF, Sabatier D, Leger P, Chaumeil P, Scotti-Saintagne C, Frigerio JM, Scotti I, Franc A and Petit RJ. 2019. Chloroplast DNA variation in a hyperdiverse tropical tree community. *Ecol. Evol.* 9: 4897 – 4905.
- [23] Chiang TY and Schaal BA. 2000a. Molecular evolution of the *atpB* – *rbcL* noncoding spacer of chloroplast DNA in the moss family Hylocomiaceae. *Bot. Bull. Acad. Sinica* 41: 85 – 92.
- [24] Chiang TY and Schaal BA. 2000b. Molecular evolution and phylogeny of the *atpB* – *rbcL* spacer of chloroplast DNA in the true mosses. *Genome* 43(3): 417 – 426.
- [25] Shaw J, Shafer HL, Leonard OR, Kovach MJ, Schorr M and Morris AB. 2014. Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. *American J. Bot.* (11): 1987 – 2004.
- [26] Liu F, Zhao SY, Li W, Chen JM and Wang QF. 2010. Population genetic structure and phylogeographic patterns in the Chinese endemic species *Sagittaria lichuanensis*, inferred from cpDNA *atpB* – *rbcL* intergenic spacers. *Bot.* 88: 886 – 892.
- [27] Abdel-Latif A and Osman G. 2017. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods* 13(1): 1 – 9.
- [28] Chiang TY, Schaal BA and Peng CI. 1998. Universal primers for amplification and sequencing a noncoding spacer between the *atpB* and *rbcL* genes of chloroplast DNA. *Bot. Bull. Acad. Sinica* 39: 245 – 250.
- [29] Sanger F, Nicklen S and Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74(12): 5463 – 5467.
- [30] Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Series* 41: 95 – 98.
- [31] Thompson JG, Higgins DG and Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673 – 4680.
- [32] Susanto AH and Dwiati M. 2019. Genetic difference between two phenotypically similar members f Asteraceae by the use of intergenic spacer *atpB* – *rbcL*. *Biosaintifika: J. Biol.Biol. Edu.* 11(3): 393 – 399.
- [33] Susanto AH and Dwiati M. 2020b. Molecular characteristics of two phenotypically identical species of Asteraceae based on the intergenic spacer *trnT* (UGU) – *trnL* (UAA). *Biodiversitas* 21(11): 5164 – 5169.
- [34] Mort ME, Crawford DJ, Archibald JK, O’Leary TL and Santos-Guerra A. 2010. Plant DNA barcoding: a test using Macaronesian taxa of *Tolpis* (Asteraceae). *Taxon* 59(2): 581 – 587.
- [35] Gong W, Liu Y, Chen J, Hong Y and Kong HH. 2016. DNA barcodes identify Chinese medicinal plants and detect geographical patterns of *Sinosenecio* (Asteraceae). *J. Syst. Evol.* 54(1): 83 – 91.
- [36] Vitales D., Feliner GN, Valles J, Garnatje T, Firat M and Alvares I. 2018. A new circumscription of the Mediterranean genus *Anacyclus* (Anthemideae, Asteraceae) based on plastid and nuclear DNA marker. *Phytotaxa* 349(1): 1 – 17.
- [37] Widodo P, Chikmawati T and Kusuma YWC. 2019. Placement of *Syzygium boerlagei* (Merr.) Govaerts (Myrtaceae) confirmed with *atpB* – *rbcL* intergenic spacer. *Biotropia* 26(1): 9 – 15.