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Failed PCR of *Ganoderma* type specimens affects nomenclature

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ABSTRACT

The nomenclature of *Ganoderma* used as a Chinese medicine is debated. A group of researchers could not amplify the DNA of type specimens and concluded the DNA was degraded irreparably. New topotypes were used as the type specimens which was premature. The use of internal amplification controls is recommended to determine if other factors were involved as alternative explanations.

Species nomenclature of the fungus *Ganoderma* defines, *inter alia*, taxa (a) used for Chinese medicines (CM) (Paterson, 2006), (b) which produce particular metabolites (Paterson, 2006) or (c) that are plant pathogens (Muniroh et al., 2014; Paterson, 2007a). DNA characters are crucial for naming fungal taxa, and determining whether names based on morphological characters are accurate. This is reflected in the emphasis placed on these by Cao et al. (2012), Yao et al. (2013) and Zhou et al. (2015), where the name of the *Ganoderma* used as CM is debated.

However, Yao et al. (2013) could not obtain results from DNA analyses of the holotype, paratype and an authentic specimen of *Ganoderma sichuanense* – the name the authors gave to the CM, although it is well known that type specimens are crucial for fixing names. They isolated novel specimens as topotypes from which they managed to obtain DNA data and considered these as representative. The conclusions they arrived at, from not being able to amplify the DNA of the original types, have implications for other organism such as plants, as the types of these specimens are also held in herbaria or collections.

Yao et al. (2013) state that (a) repeated DNA amplifications were unsuccessful because of the "condition of the specimen", (b) attempts to amplify the holotype failed owing to "the quality of DNA", and (c) experiments to amplify DNA fragments from holotype, paratype and authentic specimens of *G. sichuanense* were unsuccessful: They state, "Apparently, DNA has not been well preserved in these specimens compared with that in topotypes of *G. sichuanense*". The authors did not provide supporting data that the DNAs were damaged, such as appearance on gels, spectroscopic characteristics and DNA melting curves. In general, future researchers cannot use the types for DNA analysis in nomenclature if the DNA is completely degraded and such statements need to be made carefully and with evidence. It implies that greater effort is required to determine how best to preserve specimens for DNA analysis. In fact all is not lost.

A failed PCR may reflect inhibition of the DNA polymerase used in the reaction, problems with the reagents and/or a faulty thermal cycler (Paterson, 2007b). The problem may not be degraded DNA. *Ganoderma* is well known to produce biologically active metabolites and indeed some of these (e.g. cerebrosides and terpenoids) are DNA polymerase inhibitors (Paterson, 2008). Yao et al. (2013) could have tested their extracted DNA with internal amplification controls (IAC) (Paterson, 2007b) to assess these possibilities. IAC are additional DNA or primers added to the reaction vessels that provide another amplicon, together with the target DNA, if all conditions are satisfactory. There is a problem with (a) the PCR reagents, (b) the thermal cycler and/or (c) polymerase inhibition if the IAC amplicon is not produced. Finally, the DNA of the specimen is probably damaged if an IAC amplicon is produced, but there is not one from the specimen and novel type-specimens will be required. Of course, a crucial indication that the DNA was undamaged in Yao et al. (2013) and that the other possibilities occurred, is that Cao et al. (2012) and Zhou et al. (2015) successfully amplified the holotype, and hence was acceptable for its intended purpose as a type specimen. These authors refer to the CM as *Ganoderma lingzhi*, clearly separate from *G. sichuanense*. In conclusion, it is necessary to determine whether the other factors are involved in PCR failure before claiming that the DNA of important specimens is damaged, and the interpretations of Yao et al. (2013) are undermined as discussed herein.

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