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MAXENT MODELLING OF THE POTENTIAL DISTRIBUTION OF *GANODERMA LUCIDUM* IN NORTH-EASTERN REGION OF ROMANIA

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Abstract: *Ganoderma lucidum* is one of the most valued mushrooms in the World, because of its medicinal properties. In the context of North-Eastern Region's development, any forest product could have a valuable contribution. Therefore, it is important to understand the mushroom's ecology and generate a map of its optimal distribution. For this, we used one of the most performant species distribution models available – Maxent, field occurrences and climatic-topographic-biotic variables. After multi-collinearity testing and step-wise Maxent modelling, we came to an end with a 0.8 final model based on two predictors. Thus, in the region, the optimal habitat distribution is found in oak, beech, riparian or mixed forests bellow approximately 800 m altitude. The species can be found in almost all forests across lowland, colline and submontane regions according to tree host presence. The approach could be promising for other fungal species for the sustainable development of the region.

Keywords: *Ganoderma lucidum*, Maxent, Romania, species distribution modelling, suitable habitat.

Introduction

Ganoderma lucidum is one of the most known medicinal fungus in the World, being used for its immunomodulatory, anti-inflammatory, antiviral, antioxidative, antiaging and antitumor properties [BARBIERI & al. 2017].

Ganoderma lucidum (Photo 1) belongs to the cosmopolitan genus *Ganoderma* [RYVARDEN, 1991], Ganodermataceae family of Polyporales order, within Agaricomycetes class of Basidiomycota phylum [Index Fungorum 2016, http://www.indexfungorum.org/]. The sporocarp is perennial, having the cap and the stem inseparable. The kidney-shaped cap can reach up to 20 cm in diameter. Its colour is red to reddish brown when mature, but has bright yellow and white zones toward the margin. It has whitish zone of pores which becomes dingy brownish in age. The stem grows side to cap, rarely in a central position and can reach 12 cm in length and 3 cm thick. The spore print is brown. The spores have 8-11 x 6-8.5 µm. Setae and cystidia are absent and the hyphal system is dimitic [SĂLĂGEANU & SĂLĂGEANU, 1985; TĂNASE & MITITIUC, 2001; TĂNASE & al. 2009]. *G. lucidum* is found all over the World, growing on multiples hosts [BARBIERI & al. 2017]. In Europe, it grows on living trees or stumps of oaks or chestnuts, rarely on coniferous trees [BERNICCHIA, 2005; GERHARDT, 1999; EYSSARTIER & ROUX, 2013; SĂLĂGEANU & SĂLĂGEANU, 1985].

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Listed as the most appreciated superior tonics in Chinese traditional medicine, *G. lucidum* was used also for its benefit effects on preventing aging, tonifing the heart and improving memory [NAHATA, 2013].

Ganoderma lucidum is rich in antioxidant components that are absorbed quickly after ingestion, it has been observed that the total antioxidant activity in the blood plasma is increasing in human subjects [HAPUARACHCHI & al. 2016]. Therefore, the production of oxygen free radicals decreased, helping anti-aging process. Also, linghzi's polysacharides are known for their antibacterial, antifungal and antiviral activities. As some researchers found, Gram-positive and Gram-negative bacteria development are inhibited by some compounds from *Ganoderma lucidum* [HAPUARACHCHI & al. 2016; KAMBLE & al. 2010; VAZIRIAN & al. 2014]. Bacteria like *Escherichia coli*, *Helicobacter pylori*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Mycobacterium tuberculosis* and fungi like *Aspergillus flavus*, *Candida albicans* – common pathogens in humans, were found to react negatively to *G. lucidum* extracts [HAPUARACHCHI & al. 2016; NWANNEKA & al. 2011]. Also, plant pathogens like *Botrytis cinerea*, *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum* were inhibited by *Ganoderma lucidum* extracts [BAIG & al. 2015; HAPUARACHCHI & al. 2016].

Photo 1. *Ganoderma lucidum* on: (**a**) oak roots (Coverca Forest, Neamț County, 2016) and (**b**) oak stump (Heltiu Forest, Bacău County, 2016).

Because of its great medicinal value, there is much interest for this mushroom – the annual global turnover exceeds 2.5 billion dollars [LI & al. 2013], the vast majority being cultivated. But as TAKSHAK & al. (2017) pointed, *G. lucidum* collected from the wild can prove also as a good diet supplement, due to its high contents of nutrients. Therefore, it is essential to understand the ecology and to delineate the optimal habitat in order to use this mushroom as a valuable wild forest product. The importance of wild fungi can be therefore defined through their development perspective [BOA, 2004], which can also be applied to this species. It is also imperative for paying also attention to protect it from excessive collecting, as other wild medicinal mushroom populations declined in some parts of the World [YUAN & al. 2015].

For this, we analyzed the species-environment relationship through species distribution modelling or SDM. This tool uses species observations and environmental properties to generate predictions across different types of environments [ELITH & LEATHWICK, 2009].

One of the best prediction accuracies in spatial distribution analyses is given by Maxent or maximum entropy model, developed by PHILLIPS & al. (2006). It is a self-contained Java application which uses occurrence records and environmental variables for the study area [PHILLIPS & al. 2017]. Therefore it belong to non-parametric statistical group of methods, being different from other methods such as regression methods or profile methods. The method used by Maxent is about finding the distribution which has the maximum entropy [PHILLIPS & al. 2017]. It was chosen in this study because it outperforms other distribution models at small spatial record numbers [VAN GILS & al. 2012].

Materials and methods

The study area is North-Eastern Region in Romania. We chose this surface area in order to provide an insight of the one of the World's most valuable wild product in terms of medicinal applications [MONEY & al. 2016] in one of the poorest development regions in European Union [MAXIM, 2014].

We collected the data for the species occurrences in field surveys deployed in 2015-2017. Their distribution in region's space covers all counties and all broadleaved dominant or codominant types of forests (oak, beech, riparian and mixed forests). There were registered 18 occurrences of the species.

For the environmental layers, we used nineteen bioclimatic variables and altitude data downloaded from WorldClim database [HIJMANS & al. 2005] at finest resolution. For the topographic layers we used altitude and derived topographic variables (aspect - in cardinal directions classes - and slope) calculated in Quantum GIS Development Software 2.18. As the plant species has great influences on lignicolous fungi, and fungal species in general [KUTSEGI & al. 2015; SHI & al. 2013], we added a vegetation layer figuring the most common types of forests across the region. For this, we used the ICAS Forest Types Map (1997).

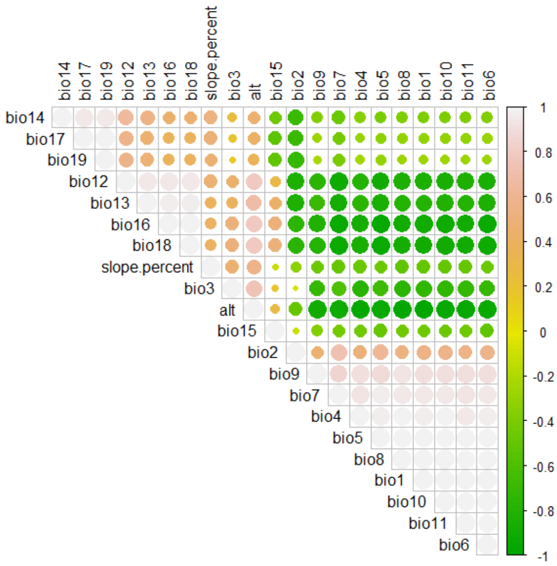

Fig. 1. Correlogram result of multi-collinearity test showing that elevation, bio15, bio19 and slope are not correlated at a 0.7 threshold.

Because multi-collinearity is a serious statistical problem in species distribution modelling and that of the large data set of environmental layers, from which some are highly correlated [CRUZ-CÁRDENAS & al. 2014], we tested the predictors using pair-wise correlation with the Spearman's rho coefficient at 0.7 threshold [MAGHIAR & STOICA, 2016].

After applying the multi-collinearity test, 4 predictors resulted as uncorrelated (Fig. 1): elevation, BIO15 (precipitation seasonality) and BIO19 (precipitation of the coldest quarter), slope (%). Those, together with qualitative variables (aspect and vegetation type) were used as predictors in Maxent modelling.

For modelling we used a machine-learning method for presence-only point data analysis called Maxent. The model estimates the probability distribution for a species in a geographic space using the maximum entropy algorithm based on species occurrences and environmental layers, known as constraints [PHILLIPS & al. 2017]. Having a high prediction accuracy [PHILLIPS & DUDÍK, 2008], it has been used for different purposes throughout researches, from conservation planning and exploring expanding distributions of invasive species to diversity predictions and endemism patterns [ELITH & al. 2010].

Using random selection 10,000 background points were generated according to geographic coordinates. The full dataset was k-fold partitioned into 75% training and 25% testing dataset.

In order to estimate the performance of the model, we used Area Under the Receiving Operator Curve (AUC). It takes values from 0 (lowest performance with random prediction) to 1 (best performance with perfectly fit prediction), [QIN & al. 2017]. A number of 10 runs were set for model building. In order to improve the model, we removed the least contributing predictors (shown by the Jackknife test) until AUC reached the minimum value of 0.8 [VAN GILS & al. 2012]. From those models, the model with highest AUC value was considered as best performer [YANG & al. 2013; YUAN & al. 2015].

For variable importance for each of the step-wise model, we used the Jackknife procedure. This test calculates the highest gain when the variable is used in isolation and the most decreasing gain when the variable is omitted. Furthermore, we removed the variables that contributed less than 5% to model accuracy gain [MOREHOUSE & TOBLER, 2013].

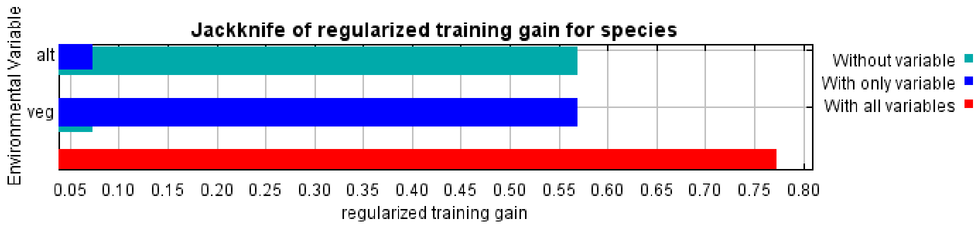

Fig. 2. The Jackknife test of final Maxent model for evaluating the relative importance of environmental variables for *Ganoderma lucidum* in North-Eastern Romania (''veg'' is vegetation type, ''alt'' is elevation)

In the final distribution map, we regrouped the values in four classes of habitat suitability: a. optimum habitat (>0.6); b. suitable habitat (0.4–0.6); c. least suitable habitat (0.2-0.4); d. unsuitable habitat (<0.2), as proposed by QIN & al. (2017). We also obtained a species optimal distribution, as a binary map delineated by 0.6 value [QIN & al. 2017].

Finally, we used the forest vegetation layer to filter out areas without forests as these do not support a suitable environment for wild populations of *G. lucidum*.

All the statistical analyses and Maxent modelling were done in the program R [R Development Core Team, 2009]. For Maxent modelling we used *dismo* package within R software.

Results and discussion

The final Maxent model provided a good accuracy, with a testing AUC value of 0.807 (Tab. 1). There is little difference from the first model (0.766) because the variables that contributed to the first and were omitted from the last had little contribution (12.2%). An interesting insight offers the aspect (in classes) which maintains a good contribution over the first 3 models but backwards under 1% in the first over 0.8 AUC model. Even if the final model have a lesser testing AUC than the 4th model, the only variables that contributes over 5% to model accuracy are altitude and vegetation type. From the first model, the percent contribution of elevation, respectively vegetation type are increasing with approximately same amount ~12%, respectively 15%, which is the approximately amount of the other non-semnificative predictors.

Tab. 1. Model's performance indicator AUC and predictor contribution in step-wise Jackknife test, run for the highest testing AUC model

model	training AUC	testing AUC	vegetation contribution (%)	elevation contribution (%)	other variables contribution (cumulated, %)	only > 5% variables contribution
1st (6 variables)	0.848	0.766	61.2	26.6	12.2	aspect 9.6%
2nd (5 variables)	0.848	0.766	61.2	26.5	12.3	aspect 9.7%
3rd (4 variables)	0.845	0.763	63.3	26.4	10.3	aspect 9.9%
4th (3 variables)	0.819	0.857	70.3	29.2	0.5 (aspect only)	-
final (2 variables)	0.836	0.807	70.6	29.4	0 (no other variable)	-

Vegetation type and elevation were part of the final model. The most contributive predictor, both as isolate and as omitted is vegetation type, confirming the strong relations existing between trees and the fungal species.

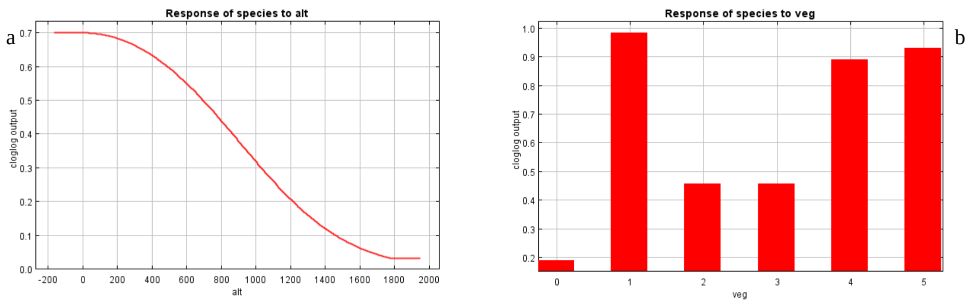

Fig. 3. Maxent taxa response curves shape for elevation (**a**) and for vegetation type (**b**); Y axis: Probability of presence; X axis: (**a**) Altitude (m) and (**b**) Vegetation type: "0" is no forest vegetation, "1" is other forests, "2" is mixed forests, "3" is coniferous forests, "4" is oak forests, "5" is beech forests.

The response curves of presence probability to predictors (Fig. 2) shows a typical response and a specific one. As the elevation grows, the presence of *Ganoderma lucidum* is decreasing in probability, thus showing a classic example of elevation-dependent taxa (Fig. 4). The curve shows a small decreasing trend at the beginning because of the association of oak and lowland riparian forests with low elevations (under 500 m). Then, the curve presents an accelerated downward trend because of the forest shift from oak to beech and mixed forests. At the end of the response curve, the trend decelerated because of the low/no-presence probability of *G. lucidum* in coniferous forests.

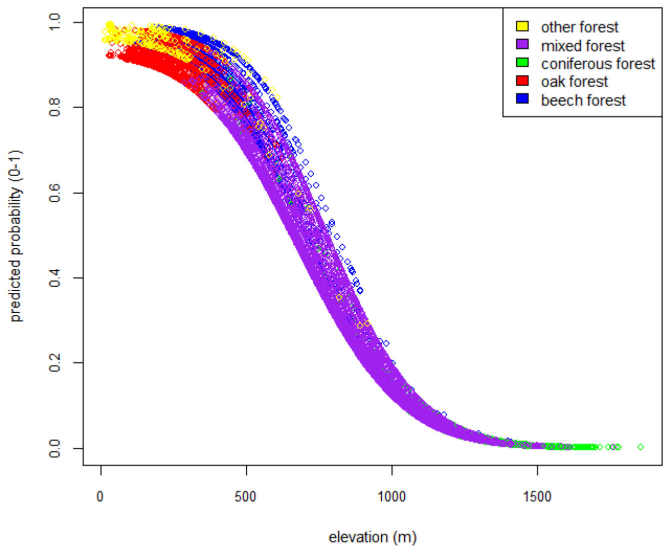

Fig. 4. The relation between *G. lucidum* predicted probability and elevation (m). The points are colored according to their forest vegetation type (5 forest classes) association.

Because of the correlation with important ecological variables, like mean annual temperature (MAT) and mean annual precipitations (MAP), this geographic distribution is also correlated with those predictors. As the temperature grows and precipitations decreases, the chance to find *G. lucidum* in the respective location also grows. The environmental space defined by MAT and MAP (Fig. 5) shows that the most suitable conditions for finding *G. lucidum* (over 60% presence probability) are places within the lowest third of MAP difference and within the highest third of MAT difference.

MAT and MAP have been found as predictors for white rot decomposers diversity at global scale [TEDERSOO & al. 2014]. The affinity of *G. lucidum* for warmer areas is confirmed by Ellenberg indicator values for MAT: 6 which corresponds to an intermediate class between fairly warm and warmth conditions, from submontane to colline and lowland sites [SIMMEL & al. 2017].

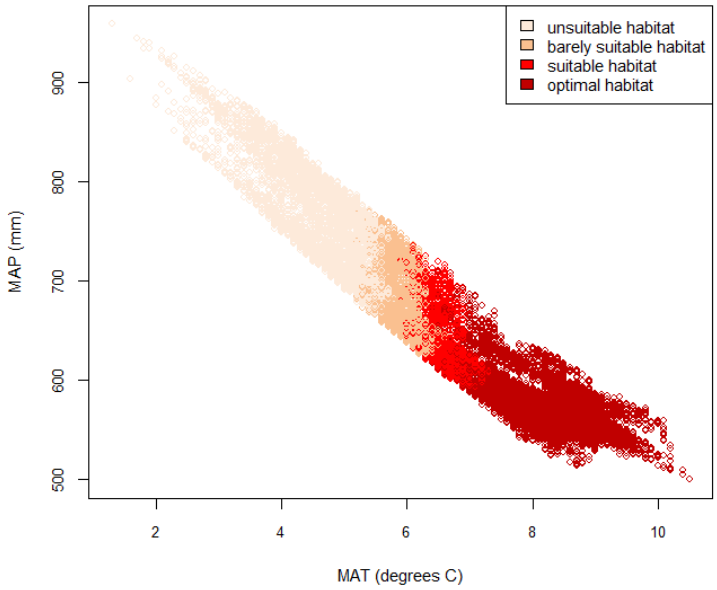

Fig. 5. A two-dimensional perspective of *Ganoderma lucidum* environmental space, defined by mean annual temperature (MAT) and mean annual precipitations (MAP). Red areas represent optimal habitat of the species according to Maxent prediction probabilities thresholded at 0.6.

This can be related to the spatial distribution of the most important predictor: forest vegetation type (Fig. 2).

The optimal suitability according to forest vegetation surfaces is divided among forest types (Fig. 6). The highest probability of presence (in terms of associated pixels) is in "other forests" class, dominated by riparian forests (100%). It is followed by oak dominated forests (99.2%) and beech dominated forests (94.2%). The least relative surfaces with optimal habitat belongs to coniferous forests (under 2%). Mixed forests takes approximately 15% of the optimal habitat, because of the elevation limitation.

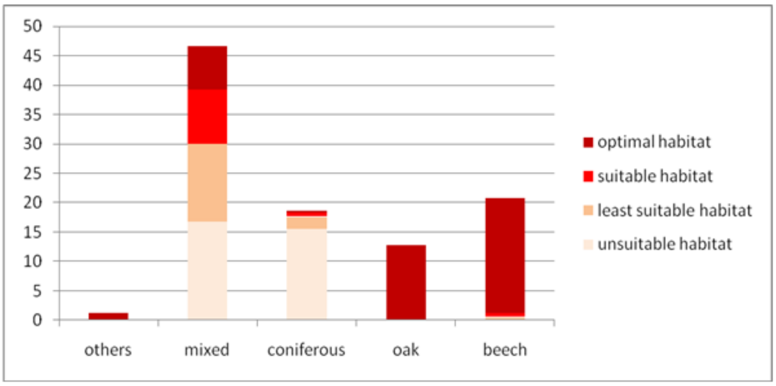

Fig. 6. Forested area (% to total) suitable for *Ganoderma lucidum* in North-Eastern Romania. Beech and oak forests represents the vast majority of optimal habitat.

The final model shows a widespread potential distribution across North-Eastern Region of Romania (Fig. 7). From an economical perspective, rural communities from all counties can access this resource.

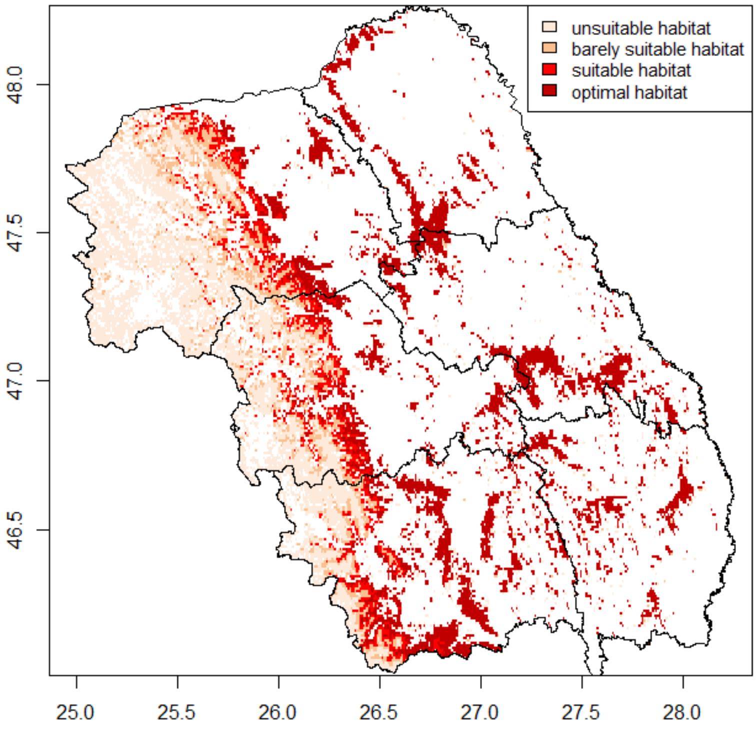

Fig. 7. *Ganoderma lucidum* habitat suitability map according to Maxent presence probability prediction.

As the mushroom's presence is highly predicted by the forest type, especially oak and beech ones, it is enough for the local people to engage in gathering this resource. A more complicated situation is in the Carpathians, where the suitability of the habitat is departing among different levels of elevation, in the presence of broadleaved forests.

The second purpose of this paper was not only to predict areas with *G. lucidum* for financial collecting but also for its protection. Over-collecting could affect wild *G. lucidum* populations. Therefore it is essential to find robust conservation measures that can be applied for effective conservation management.

Conclusions

The potential distribution in geographical space of *Ganoderma lucidum* was accurately predicted through Maxent modelling, having a 0.806 testing AUC. By applying multiple predictor filtering, overfitting was removed and non-important predictors were not included in the final model. The species distribution was influenced by the variation of elevation, as in other classical environmental-species relationship. But the most important predictor was the forest vegetation type, *G. lucidum* having the optimal habitat in oak and beech forests. Those forests have a good covering across North-Eastern Romania, therefore the mushroom can be efficiently exploited through sustainable development.

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