Faculty of Biology

Jagiellonian University


Methodological Workshop in Evolutionary Biology

for PhD Students – practical part

Online course, 16-29th September 2021

Preparation of the compendium: Aleksandra Żmuda

Photos: Joanna Rutkowska

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# List of participants

## Organiser

| | | | |
|---|---|---|---|
| Dr hab., prof. UJ | Joanna | Rutkowska | Institute of Environmental Sciences, JU |

## Guest lecturer

| | | | |
|---|---|---|---|
| Dr | Małgorzata | Łagisz | School of Biological, Earth and Environmental Sciences, UNSW |

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| Dr hab. | Hajnalka | Szentgyörgyi | Institute of Botany, JU |
| Dr hab. | Aleksandra | Walczyńska | Institute of Environmental Sciences, JU |
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## Participants

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| MSc | Agata | Burzawa | Institute of Environmental Sciences, JU |
| MSc | Junchen | Deng | Institute of Environmental Sciences, JU |
| MSc | Pritam | Dey | Institute of Zoology and Biomedical Research, JU |
| MSc | Paulina | Jośko | Institute of Zoology and Biomedical Research, JU |
| MSc | Maëlle | Lefeuvre | Institute of Environmental Sciences, JU |
| MSc | ChuChu | Lu | Institute of Environmental Sciences, JU |
| MSc | Monika | Opałek | Institute of Environmental Sciences, JU |
| MSc | Monika | Ostap-Chęć | Institute of Environmental Sciences, JU |
| MSc | Filip | Turza | Institute of Environmental Sciences, JU |
| MSc | Aleksandra | Żmuda | Institute of Botany, JU |

# Research topics

## Topics proposed by participants

- In what taxa is temperature-size rule studied? (AB)
- Is there a gender bias among authors of biological papers? (MO)
- What are the methods of yest modifications in ale beer? (MO)
- What aspects of maternal effects are studied in birds? (ML)
- Plastic concentration in natural environment (ML)
- What do we know about cognition in songbirds? (CL)
- Distribution of animal personality research (CL)
- Popularity of honeybee as a model species (MO-Ch)
- Effectiveness of bee products (propolis) in medicine (MO-Ch)
- Pesticide and habitat loss biodiversity (AB)
- Artificial light effects on birds ecology/fitness (JD)
- Diversity of symbionts of insects (JD)
- Fungal infections (*Batrachochytrium*) in amphibian decline (PJ)
- Influence of mitochondrial introgression on animal fitness (PJ)
- Catalog of factors determining ecological success of invasive species (FT)
- Environmental and economic aspects of invasive species (FT)
- Different ways of anti-mite treatment of honeybees including timing, location & year of publication (AŻ)
- Pollinators hotels - materials, species, localities (AŻ)
- Reproductive modes and cryptobiotic ability effects on the dispersal in meiofauna (PD)
- Inclusion of keystone species in conservation strategies in ecosystems of different types (PD)

## Topics selected by participants

- Environmental and economic aspects of invasive species (FT)
- Fungal infections (*Batrachochytrium*) in amphibian decline (PJ)
- Pollinators hotels - materials, species, localities (AŻ)
- Popularity of honeybee as a model species (MO-Ch)

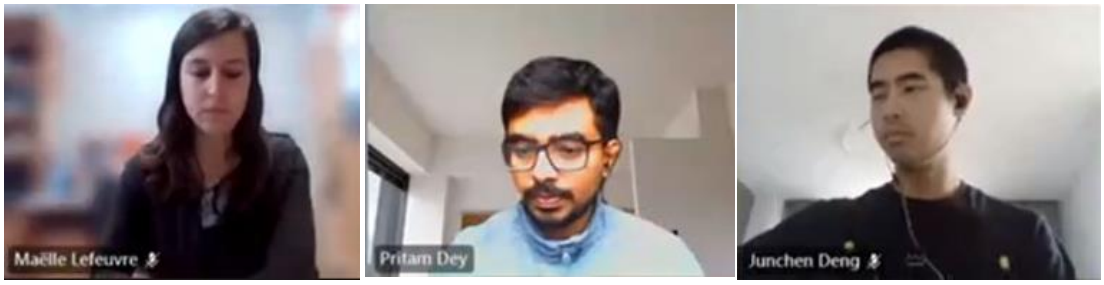
## Topics finally developed by participants

- How invasive species get notorious -- a systematic mapping on the factors that contribute to their ecological success
- Facing worldwide amphibian decline: A systematic review of fungal (*Batrachochytrium spp.*) infection patterns in natural populations
- Systematic mapping of the current trends in animal personality research
- Bee a science star – a systematic review of the primary research domains on *Apis mellifera*

# How invasive species get notorious -- a systematic mapping on the factors that contribute to their ecological success

by

Maëlle Lefeuvre, Pritam Dey, Junchen Deng



Study plan

# Which factors determine the ecological success of invasive animal species?

Maëlle Lefeuvre, Pritam Dey, Junchen Deng

**Aim of the study**:
Invasive species often represent real threats to native species. Their high adaptiveness often allows them to establish, reproduce and/or spread faster than native species, which could lead to population decline or even the extinction of native species. In this study, we aim to categorize the environmental and ecological factors that contribute to the ecological success of invasive species, in particular, invasive animals, through systematic mapping of the relevant studies in the last three years (2019-2021). We aim to answer the following questions: 1) how diverse are the invasive animal species? 2) which factors favour the success of invasive animals over native species?

**Scope of the study**:
- *Population*: invasive animals
- *Intervention*: factors favouring the success of invasive species. 1) environmental factors, such as climate, food availability and predation; 2) biological factors, such as genetics, immunity to pathogens or diseases and reproductive rate.
- *Comparison*: None
- *Outcome*: the ecological success of invasive species, such as the increase in population size, range expansion and the increase in reproductive rate.
- *Study type*: experimental, correlative or theoretical

**Search Terms and Strings:**
We extracted several search terms from the PICOS (Population, Intervention, Comparison, Outcome and Study type) framework. From "Population", we selected invasi*. From "Outcome", we selected "ecolog* success", increas*, population*, expan*, range, success and reproduc*. The asterisk (*) is a "wildcard" that represents any group of characters, including no character. The terms from "Population" and "Outcome" will be linked with the boolean operator "AND" in the search string. The terms within each category will be linked with the boolean operator "OR" and the proximity searching operator, "W/3" in Scopus or "NEAR/3" in Web of Science, which searches the text that has the two linked terms within a distance of three characters.
To get the proper amount of research papers that fits this study, we limited the publication year to the last three years, i.e. 2019, 2020 and 2021. We also limited the language to "English". In case of the inclusion of only invasive animals, we noticed the difficulty to filter out invasive plants by excluding the terms, such as "grass", "weed" and "tree", because many animals, such as grasshoppers, have words related to plants in their name. Thus, we decided to exclude species other than animals not in the search strings but in the paper screening in the following steps. The following are two examples of search strings in the Scopus database and Web of Science Core Collection.

Search String in Scopus
ABS ( invasi* AND ( "ecolog* success" OR ( increas* W/3 population* ) OR ( expan* W/3 range ) OR ( success W/3 reproduc* ) ) ) AND ( LIMIT-TO ( PUBYEAR , 2021 ) OR LIMIT-TO ( PUBYEAR , 2020 ) OR LIMIT-TO ( PUBYEAR , 2019 ) ) AND ( EXCLUDE ( DOCTYPE , "re" ) ) AND ( LIMIT-TO ( LANGUAGE , "English" ) ) → **1004 records**

Search String in Web of Science

invasi* AND ("ecolog* success" OR (increas* NEAR/3 population*) OR (expan* NEAR/3 range) OR (success NEAR/3 reproduc*)); Refined by: Abstract, Pub year: 2019 - 2021, Review Articles: Excluded, Languages: English. → **957 records**

**Inclusion criteria for the studies:**

In both Web of Science and Scopus, we searched for suitable articles using the search strings presented in this document. Both Web of Science and Scopus allow for exclusion of the reviews and English publications from the results, and this option will save us time during later exclusions. We saved the results and used the software Rayyan to filter our list of articles.

The term "invasive" is our main keyword for our research, however it does not refer only to species. This also characterizes the methods (invasive and non-invasive) which can be used in various measurements. Thus, we will include "invasive species" as keywords for inclusion and "invasive method" and "non-invasive" as keywords for exclusion.

Keywords for inclusion

We are interested in the factors which contribute to the establishment and spreading of invasive species. Thus, the related terms can help us include some articles. Those terms will include: "environmental factors", "ecology", "ecological success", "reproductive success", "expansion range".

Keywords for exclusion

The term "invasion" was included by our research string but this term is often used in medicine when talking about diseases such as cancer. So we will try to remove as many medical publications with exclusion terms as possible. To do so, we can use the keywords, such as "cell", "cancer" and "disease" to exclude articles in the field of medicine.

In our systematic review, we will focus on invasive animal species. Thus, papers reporting plant or microorganism species should be removed. To do so, we will use keywords, such as "plant", "grass", "weed", "tree", "algae", "microorganism", "micro-organism" and "fungi", for articles exclusion.

**Protocol for data collection from the full texts**

Here is our decision tree (Fig. 1) to exclude all irrelevant publications from our research results. Reviews, articles published before 2019 and articles in other languages than English have already been removed at the research level.

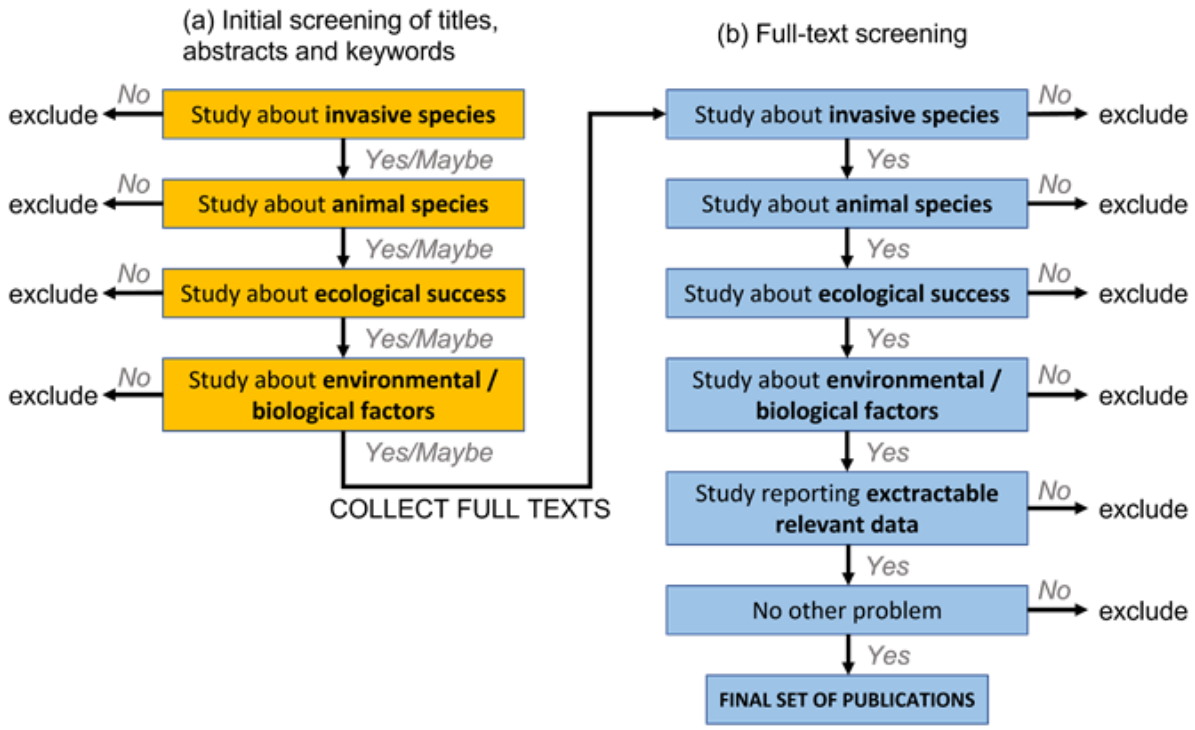



Figure 1: Decision tree for the filtering of our list of publications, at the abstract level (yellow boxes) and at the full-text level (blue boxes).

Report - first version

# Which factors determine the ecological success of invasive animal species?

Maëlle Lefeuvre[1], Pritam Dey[2], Junchen Deng[1]

[1] Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland
[2] Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

## ABSTRACT

The invasion of exotic species often puts a great threat to the local ecosystem. The high adaptiveness of invasive species often enables them to establish, reproduce and/or spread faster than native species, which could lead to local native species decline or even extinction. Various factors, such as climate, behaviour, and genetics, could contribute to the ecological success of invasive species. Understanding these factors are crucial for the management of invasive species and the protection of the local ecosystems threatened by these species. In this study, we categorized the environmental and ecological factors that contribute to the ecological success of invasive animal species, through systematic mapping of the relevant studies in the last three years (2019-2021). Our search identified 1062 articles through the searching strings and only 107 papers were included in this study after the full-text screening. We found that a large number of studies focus on the taxa Arthropods, the Range Expansion as ecological success and factors such as Climate, Genetics and Physiological traits. This result indicates a focus of recent studies on the impact of invasive species on humans and the development of invasive populations in the future.

**Keywords**: invasive species, animal, ecological success, systematic review

## INTRODUCTION

The invasion process is considered as a sequence of steps leading to the persistent presence of a species in an area that it has never occupied before [1]. This sequence describes the biological barriers the species have to overcome to successfully establish, reproduce and spread to new territories. The species that complete the entire invasion process are highly adaptive, and often represent a concrete threat for native species and their associated ecosystems. The devastating consequence of species invasion has been well documented worldwide. For example, introduced predators have contributed to 58% of the extinction of mammals, birds and reptiles, especially on islands [2]. Invasive species are also the cause of important ecosystem changes in areas preserved from high anthropogenic pressure [3]. In addition, some non-native species directly impact human health as disease vectors or human economy through the cost of species management and decrease in the supply of food and other products [4,5]. Solving the issues of invasive species can be crucial for both humans and nature.

An important step in the management of non-native species is to understand the mechanisms behind the successful invasion. Human activities, such as trade and transport, are often the primary drivers for the quick spread of invasive species to a distant area [6]. Global climate change may improve the habitability of certain areas and favour the invasion of non-native species [7]. When establishing in a new habitat, invasive species often possess biological advantages that help them to outcompete native species. Some invasive species are free from the natural enemies (e.g. parasites) they often encountered in their previous ecosystem (Enemy Release Hypothesis) [8], others may carry new parasites or diseases to which local species are not immune (Novel Weapons Hypothesis) [9]. Understanding these potential mechanisms could facilitate the future implementation of strategies in managing and controlling the invasive populations.

In this study, we categorized the environmental and biological factors which contribute to the ecological success of invasive species, in particular, invasive animals, through systematic mapping of the relevant studies in the last three years (2019-2021). We aimed to answer the following questions: 1) how is the distribution of the studied animals in common taxa, and 2) which factors favour the success of invasive animals over native species.

## METHODS

### Components of the primary question

- *Population*: invasive animals
- *Intervention*: factors favouring the success of invasive species, which can be classified into
  1) environmental factors, such as climate and resource availability;
  2) biological factors, such as genetics, behaviour and physiological traits.
- *Comparator*: None
- *Outcomes*: the ecological success of invasive species, such as the increase in population size and range expansion.

### Search Terms and Strings

We decided to focus on invasive animal species, excluding plants and microorganisms. As it was impossible to include in our search all animal taxa and the corresponding common and latin names of each animal that could appear in the title or the abstract of the articles, we simply used invasi* as a global search term to include all studies about invasive species. We then defined ecological success as an increase of the population or an expansion of the occupied range. To cover these notions, we integrated the terms "ecolog* success", increas*, population*, expan*, range, and success and reproduc*, as reproductive success often leads to the growth of the population. The asterisk (*) is a "wildcard" that represents any group of characters, including no character. The terms invasi* was linked to the other terms with the boolean operator "AND" in the search string. The terms within each category were linked with the boolean operator "OR" and the proximity searching operator, "W/3" in Scopus or "NEAR/3" in Web of Science, which searches the text that has the two linked terms within a distance of three words.

To get the proper amount of research papers that fits this study, we limited the publication year to the last three years, i.e. 2019, 2020 and 2021. We also limited the language to "English" and conducted our search in only two online databases, Scopus and Web of Science. In case of the inclusion of only invasive animals, we noticed the difficulty to filter out invasive plants by excluding the terms, such as "grass", "weed" and "tree", because many animals, such as grasshoppers, have words related to plants in their name. Thus, we decided to exclude species other than animals not in the search strings but in the paper screening in the next steps. The following are two examples of search strings in the Scopus database and Web of Science Core Collection:

Search String in Scopus (1004 records extracted)

ABS ( invasi* AND ( "ecolog* success" OR ( increas* W/3 population* ) OR ( expan* W/3 range ) OR ( success W/3 reproduc* ) ) ) AND ( LIMIT-TO ( PUBYEAR , 2021 ) OR LIMIT-TO ( PUBYEAR , 2020 ) OR LIMIT-TO ( PUBYEAR , 2019 ) ) AND ( EXCLUDE ( DOCTYPE , "re" ) ) AND ( LIMIT-TO ( LANGUAGE, "English" ) )

Search String in Web of Science (957 records extracted)

invasi* AND ("ecolog* success" OR (increas* NEAR/3 population*) OR (expan* NEAR/3 range) OR (success NEAR/3 reproduc*)); Refined by: Abstract, Pub year: 2019 - 2021, Review Articles: Excluded, Languages: English.

**Inclusion criteria for the studies:**

In both Web of Science and Scopus, we searched for suitable articles using the search strings presented in this document. Both Web of Science and Scopus allow for exclusion of the reviews and English publications from the results, and this option saved us time during later exclusions. We then used the online software Rayyan (https://rayyan.ai/) to perform abstract screening.

Keywords for inclusion

The invasive species was the main focus of this research. Keywords, such as "invasive", "invasion", "invasive species", "non-native" and "alien", were included to filter studies about invasive species. For the factors contributing to the ecological success of invasive species, the relevant keywords, such as "environmental factors", "ecological success", "population increase", "reproductive success" and "range expansion", were also included.

Keywords for exclusion

This research focused on only invasive animal species. Thus, studies about plants and microorganisms were removed by the keywords "plant", "grass", "weed", "tree", "algae", "microorganism" and "fungi". A problem with the keyword "invasive" is that it refers not only to species but also to the invasive and non-invasive methods that can be used in animal or medical experiments. Thus, we set "invasive method" and "non-invasive" as keywords for exclusion. To identify medical research that is not relevant to our interest, we used keywords such as "cell", "cancer", ''patient'' and "disease".

**Protocol for data collection from the full texts**

During the full-text screening, we extracted the following information: 1) the taxa of the invasive animals, including Mammals, Birds, Fishes, Reptiles, Amphibians, Arthropods, Molluscs and other invertebrates (e.g. earthworms, corals, planktons, star fish); 2) the types of ecological success, i.e. the increase of population size, the range expansion or both; 3) the factors contributing to the ecological success of invasive animals, including environmental factors (e.g. climate, resources availability and anthropogenic factors) and biological factors (e.g. physiological traits, genetics, behaviour and interspecific interaction). All information was collected from each publication using an online survey created with Google Forms. The final results were visualized in R v4.0.3 [10].

**RESULTS**

After removing the duplicates, we obtained 1062 articles with our two search strings. A total of 179 articles were included after abstract screening. However, a few documents were totally unavailable and only 174 full texts were screened in the second step. During the screening of full texts, we excluded 67 articles and we ended up with a dataset of 107 articles related to the factors contributing to the ecological success of invasive animal species (Fig. 1).

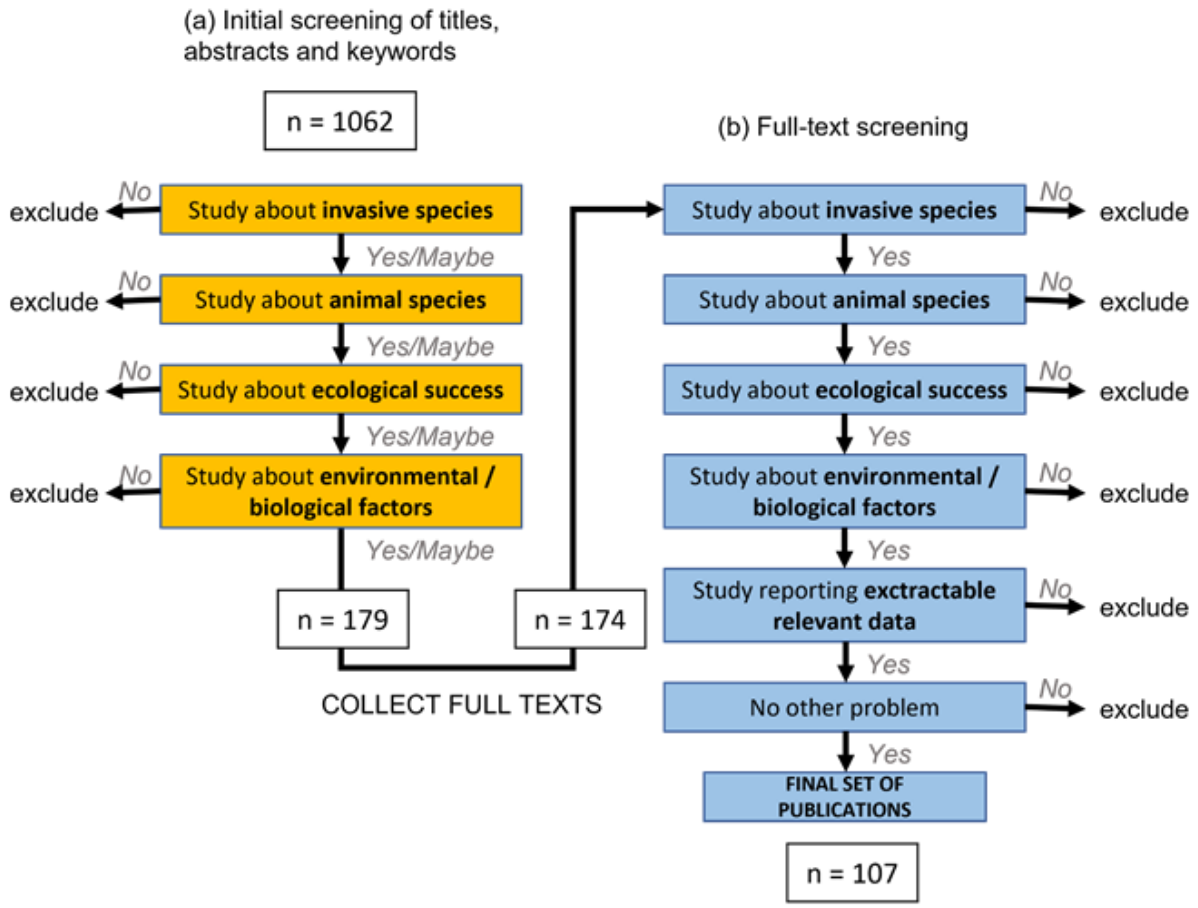



**Figure 1.** Decision tree for the filtering of our list of publications, at the abstract level (yellow boxes) and at the full-text level (blue boxes). The number of publications at each step is indicated in white boxes.

The full text screening and the information extracted from the publications reveal a clear heterogeneity of the studied taxa, the ecological success and associated factors (Fig. 2). Regardless of the ecological success feature, Arthropods are the main taxa studied, with a majority of publications on crop pests and mosquitos, all of which are closely related to humans. In terms of ecological success features, the biggest interest goes to the range expansion of alien species, either to understand their past invasion or to predict their future range. Factors explaining the invasive success of non-native animals are diverse but the light is shed mainly on Physiological traits (e.g. morphology traits, adaptation, resistance), Genetics and Climate. The studies on physiological traits aim at justifying how non-native species outperform native ones. The studies about genetics mainly trace the invasive history of alien species using population genetics tools. The studies about global climate change often predict the consequences of invasion or the future distribution of invasive species with the changing climate.

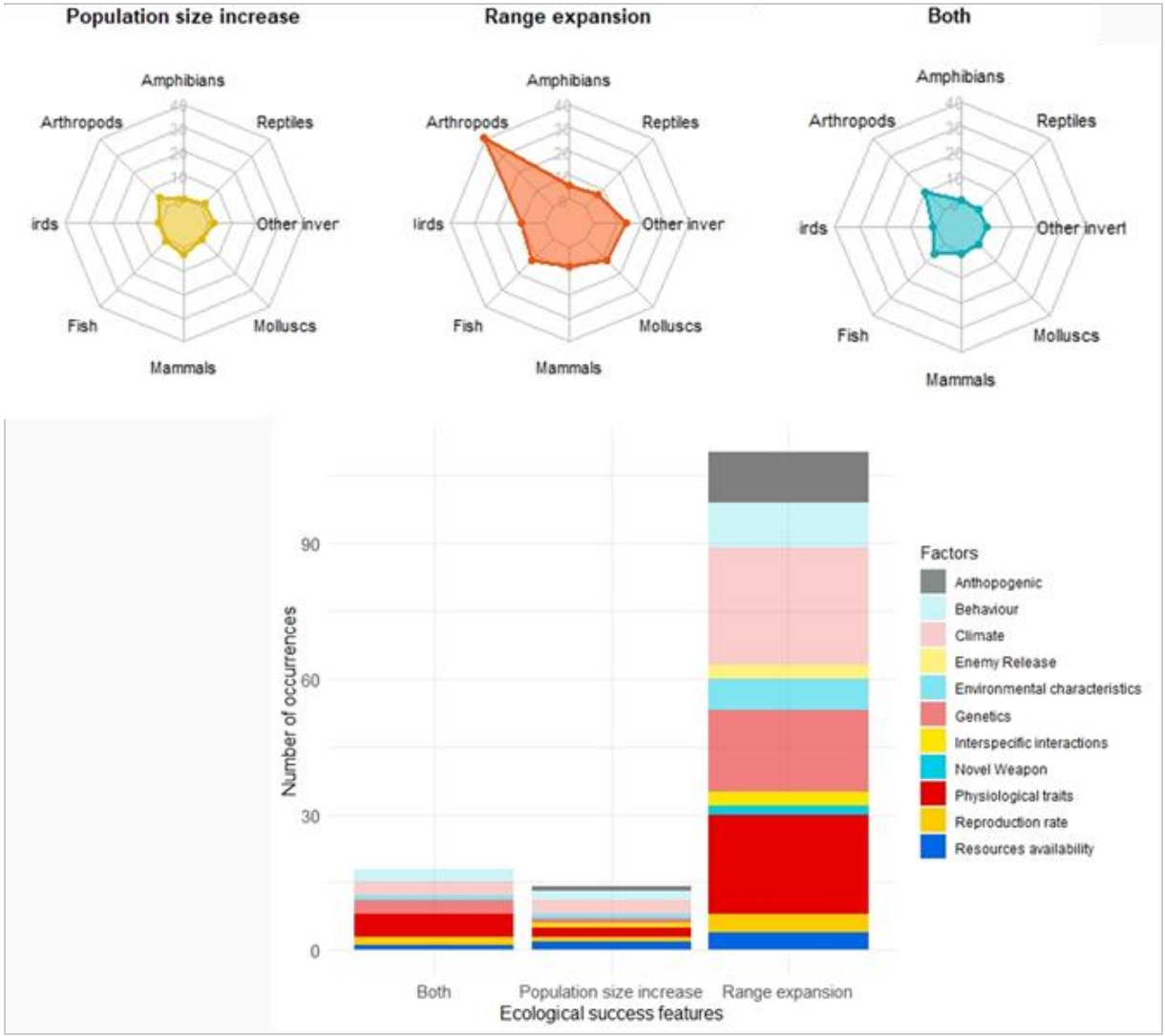



**Figure 2.** The radar charts represent, for each animal taxa, the number of publications studying the increase of population size, the expansion range or both of these ecological success features. The bar plot reports the factors investigated in publications in order to understand the invasiveness of non-native animal species. Publications could measure more than one factor, increasing the number of occurrences in this graph.

## DISCUSSION

Based on our results, we can see that research in the field of invasive animal species is mainly led by the concern about the impact of these species on human beings and societies. Some insects have been intentionally introduced in order to fight against crop pests but became themselves an issue for ecosystems, cattle or crops [5]. Some Arthropods, such as Tiger mosquitos, are also responsible for human health issues [11–13] due to the diseases they can carry and transmit. Thus, it is not surprising to see a majority of publications trying to understand the invasive mechanisms, to determine their current invasion level and propose control and management plans accordingly, and to foresee the future population development of these species. The prediction of future invading events under climate change could prepare local communities or countries for the potential risks and manage invasive species efficiently before they get rampant. This explains the over-representation of climatic factors and their effects on the expansion range of alien species in these publications.

The legitimate interest for human wellness may hide other problems created by invasive species. The low number of publications focusing on the impact of non-native animals on ecosystems and native species may underestimate the severity of the invasion. One can mention that direct effects of introduced species on non-productive ecosystems can have considerable effects on human beings [14], and these threats need as much investigation as current main topics in this field of research. In addition, different species can display very different invasive mechanisms and characteristics [15,16]. Generalizing the adaptiveness and invasiveness of the most studied species to the other species in the same taxa is inappropriate. We need to put more effort into our understanding of animal invasions to be truly able to anticipate and react in order to minimize their damages on both humans and biodiversity.

At the end, we would like to discuss the narrow scope of our systematic mapping, due to the short time period (i.e., only the last three years) and the exclusion of publications about invasive plants and microorganisms. We also excluded modelling studies that only construct models based on current knowledge yet draw accurate invasion predictions, and guidelines for alien species management which give concrete tools to communities and countries. Our results only showed the heterogeneity of this research field, without much information in other animal taxa than Arthropods, other features than range expansion, and other impacting factors than climate, genetics and physiological traits. To complete our overview of the recent publications dedicated to invasive species, introduced plants and their adaptiveness and invasiveness should be reviewed, as their impact on ecosystems is at least as dramatic as the impact of animal species [5,17].

**ACKNOWLEDGEMENTS**

The authors are thankful to Dr hab. Joanna Rutkowska *(Professor, Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland)* for her continuous support, advice, and guidance during the preparation of the manuscript. Authors also wish to thank Jagiellonian University for the necessary facilities during the data collection.

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Reviews

## Reviewer: Aleksandra Walczyńska

In the study I have a pleasure to review, the authors focus on the timely and important issue of the reasons behind the animal ”invasiveness”. In particular, in the thorough research review the authors aimed to find out which factors, environmental or biological, decide on the success of invasive animals. The strong points of the review are the study summary in Abstract, the thorough presentation of scientific background in Introduction and the very well-thought-of methodology of the literature survey. Following are my main concerns:

1. Categorization of the examined factors – my impression is that using the categories of environmental vs. biological factors are not well demarcated. For example, resources availability or anthropogenic effect are not necessarily of the environmental base. On the other hand, physiological traits is not exactly what one would select as a biological factor. Perhaps the authors could consider the alternative categorization into external (outside and organism) and internal (inside an organism) factors. Actually, the categorization could be easier if the hypotheses posed or predictions would be clearer, which makes me to approach my next doubt.
2. The aims of the study – it is possible that I am not right, but in my opinion the question 1 of the study, to find out what is the distribution of examined taxa, is not really interesting or novel. I cannot think of any important Scientific Problem this question would contribute into. Question 2 is much more interesting in this regard, but… it brings me to another concern.
3. The question 2, invoked in the project title and Introduction, is actually not addressed at all. Perhaps I missed something, but the discussion is almost exclusively devoted to the fact that the majority of the selected articles was focused on the impact on human beings and the societies and not on ecosystems. The authors seem to be quite disappointed (as I am!) but this should be somehow solved if the scientific question posed is to be addressed. In this regard, there is actually no analysis that could provide at least a partial answer to question 2. Additionally, the distinction between two examined quality measures of invasive species, population size increase and range expansion, is only showed graphically, but not analyzed and not discussed.

In general, the authors made a good and hard work in the sound literature survey but this potential was not exploited enough in the analyses.

## Reviewer: Monika Ostap-Chęć

The study provide a systematic review of the literature on invasive animal species. Authors focused on publications, which show an increase in the population size or expansion range of invasive animal species, and analyzed them in terms of the factors determining this ecological success, as well as the group to which the tested animal belongs. The factors influencing ecological success have been divided into biological and environmental. To the biological factors, authors include all aspects related to the adaptation of the organism to occupy a new terrain, such as physiological traits, genetics, behaviour and interspecific interaction. As environmental factors authors recognize climate, resources availability and anthropogenic factors.

The presented research concern an important problem nowadays, as invasive species are considered to be one of the major threats both to natural environment and human. In my opinion, these studies are innovative and provide valuable information, both for specialists in the field and a wider audience. The obtained results provide valuable information on the most commonly studied invasive animals, as well as the reasons for their easy spread.

The introduction is clearly written, understandable even to a non-specialist and provide a good justification for the validity of the performed research. The research methodology is appropriate and mostly well described. The results are clearly described and discussed. The length and structure of the text is appropriate.

Comments:

1. The title does not indicate the type of article. My suggestion is to inform the reader already at this stage that this is a systematic review.
2. For me, the abstract is not clearly written. There is no clearly defined aim of the study, which makes the sentence concerning the results not very informative.
3. In methods, parts: "Components of the primary question", "Search Terms and Strings" and "Inclusion criteria for the studies" are clearly and well described. However, in section "Protocol for data collection from the full texts" there are not mentioned all categories of factors contributing to the ecological success of invasive animals. For example, in results, on the graph there is category "reproduction rate" or "novel weapon" and they are not listed anywhere before. All analysed categories of factors should be listed in the methods along with their classification as environmental or biological, and short description. There is no information about how many people took part in abstracts and full text screening. Whether the give abstract was analyzed by one or more people?
4. The large amount of data in the graph is confusing. Radar charts and bar plot should be separate graphs with separate caption. In case of leaving it as one graph, it should be signed as A and B, both in the graph and caption.
   On the bar plot, it is unclear which factors are biological and which are environmental. It should be clearly visible. Moreover, all categories from one type (biological / environmental) should be close to each other on the bar plot and legend.
5. Different parts are written in different fonts in a document. This should be corrected

Line 47-48: the description of first aim is not clear
Line 92: non-English
Line 111: the name of the groups of animals with the lowercase
Line 117: which package?
Figure 1: What does it mean "No other problem?"
Line 130: categories of factors should be lowercase
Line 146: species names with the lowercase letter
Line 159-161: In research, model species are often used and based on them, other species are Inferred. Such a sentence would need to be supported by literature data.

## Reviewer: Filip Turza

The manuscript entitled "Which factors determine the ecological success of invasive animal species?" submitted to Biology Letters examines the potential factors determining the ecological success of invasive animal species. The study compares different factors such as anthropogenic, behavior, climate, enemy release, environmental characteristic, genetics, interspecific interactions, novel weapon, physiological traits, reproduction rate and resources availability. This study deals with the population size increase and range expansion of invasive species depending on the studied factor. It brings an important contribution to the literature by showing the large difference between factors and preseting how different factors lead to distinct effects on ecological success of invasive species. Nevertheless, there are some issues the authors need to address.

The introduction was written correctly. The research goal is 100% justified. I really liked the first paragraph of the introduction explaining the importance of studies devoted to invasive species. The authors emphasize the negative impact of these organisms on native species and ecosystems as well as on human health and the economy. I think it would be great if the authors include more numerical data. In line 30, the authors put a specific % but it is not clear what is the scale of the total number of animals.

The methods are fine, although I recommend using narrative text at the beginning of this section. I was a bit lost why the authors used dots and what components of the primary question meant. Please put information about the „PICO statement" used in the study. Moreover, I suggest creating a table for 50-53 lines. This is however not mandatory and I leave it to the decision of the authors. Secondly, authors should focus more on properly arguing their decision, e.g. why only the last three years were used in the study (I understand that there was a limited time to perform the analyzes, but I think the authors can try to provide a more scientific explanation) and why the research was limited only to animals excluding plants and microorganisms (I suggest a good argument would be to emphasize that invasive animals lead to negative effects to native species and ecosystems at a greater scale than for example plants and/or microorganisms, although this information should be verified using relevant literature).

The results answer two questions, namely 1) how is the distribution of the studied animals in common taxa, and 2) which factors favor the success of invasive animals over native species. However the title is devoted to only the latter. Please clarify it. The analysis seems correct but it is hard to follow this section seeing one big figure with several graphs. Please be more precise, maybe divide Figure 2 into panels (e.g., A, B, C) and explain what the given panel represents. This will make it easier to follow.

In the discussion, I miss the main conclusion of the results. I didn't get the information on which factors favor the ecological success of invasive species and explanations proposed by the authors. For instance, the authors mentioned the factor climate but there is still not clear why climate change determines the ecological success of invasive animal species. In the introduction section, the authors mentioned it (lines 37-38) and I suggest this information should be part of the discussion.

I congratulate the authors for an interesting systematic review and I have no doubt the authors can revise the manuscript.

Report – final version

# How invasive species get notorious -- a systematic mapping on the factors that contribute to their ecological success

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## ABSTRACT

The invasion of exotic species often puts a great threat to the local ecosystem. The high adaptiveness of invasive species often enables them to establish, reproduce and/or spread faster than native species, which could lead to local native species decline or even extinction. Various factors could contribute to the ecological success of invasive species. Understanding these factors are crucial for the management of invasive species and the protection of the local ecosystems threatened by these species. In this study, we aim to illustrate potential knowledge gaps in this field of research, and for that we categorized the environmental and ecological factors that contribute to the ecological success of invasive animal species, through systematic mapping of the relevant studies in the last three years (2019-2021). Our search strings identified 1062 articles and only 107 papers were included in this study after the full-text screening. We found that a large number of studies focus on the taxa arthropods, the range expansion as ecological success and factors such as climate, genetics and physiological traits. This result indicates a focus of recent studies on the impact of invasive species on humans and the development of invasive populations in the future.

**Keywords**: invasive species, animal, ecological success, systematic review

## INTRODUCTION

The invasion process is considered as a sequence of steps leading to the persistent presence of a species in an area that it has never occupied before [1]. This sequence describes the biological barriers the species have to overcome to successfully establish, reproduce and spread to new territories. The species that complete the entire invasion process are highly adaptive, and often represent a concrete threat for native species and their associated ecosystems. The devastating consequence of species invasion has been well documented worldwide. For example, introduced predators have contributed to 58% of the extinction of mammals, birds and reptiles, especially on islands [2]. Invasive species are also the cause of important ecosystem changes in areas preserved from high anthropogenic pressure [3]. In addition, some non-native species directly impact human health as disease vectors or human economy through the cost of species management and decrease in the supply of food and other products [4,5]. Solving the issues of invasive species can be crucial for both humans and nature.

An important step in the management of non-native species is to understand the mechanisms behind the successful invasion. Human activities, such as trade and transport, are often the primary drivers for the quick spread of invasive species to a distant area [6]. Global climate change may improve the habitability of certain areas and favour the invasion of non-native species [7]. When establishing in a new habitat, invasive species often possess biological advantages that help them to outcompete native species. Some invasive species are free from the natural enemies (e.g. parasites) they often encountered in their previous ecosystem (Enemy Release Hypothesis) [8], others may carry new parasites or diseases to which local species are not immune (Novel Weapon Hypothesis) [9]. Understanding these potential mechanisms could facilitate the future implementation of strategies in managing and controlling the invasive populations.

In this study, we categorized the environmental and biological factors which contribute to the ecological success of invasive species, in particular, invasive animals, through systematic mapping of the relevant studies in the last three years (2019-2021). We aimed to answer the following questions: 1) were invasive species studied evenly in the common animal taxa, and 2) which factors are supposed to favour the success of invasive animals over native species.

## METHODS

### Components of the primary question

The screening of articles for systematic mapping was performed following the procedure in Figure 1. We first established the PICO (**P**opulation, **I**ntervention, **C**omparator, **O**utcomes) framework to help us construct our search strings.

- *Population*: invasive animals
- *Intervention*: factors favouring the success of invasive species, which can be classified into 1) environmental factors, such as climate and resource availability; 2) biological factors, such as genetics, behaviour and physiological traits.
- *Comparator*: None
- *Outcomes*: the ecological success of invasive species, such as the increase in population size and range expansion.

### Search Terms and Strings

We decided to focus on invasive animal species, excluding plants and microorganisms. As it was impossible to include in our search all animal taxa and the corresponding common and latin names of each animal that could appear in the title or the abstract of the articles, we simply used invasi* as a global search term to include all studies about invasive species. We then defined ecological success as an increase of the population or an expansion of the occupied range. To cover these notions, we integrated the terms “ecolog* success”, increas*, population*, expan*, range, and success and reproduc*, as reproductive success often leads to the growth of the population. The asterisk (*) is a “wildcard” that represents any group of characters, including no character. The terms invasi* was linked to the other terms with the boolean operator “AND” in the search string. The terms within each category were linked with the boolean operator “OR” and the proximity searching operator, “W/3” in Scopus or “NEAR/3” in Web of Science, which searches the text that has the two linked terms within a distance of three words.

To get the proper amount of research papers that fits this study, we limited the publication year to the last three years, i.e. 2019, 2020 and 2021. We also limited the language to “English” and conducted our search in only two online databases, Scopus and Web of Science. In case of the inclusion of only invasive animals, we noticed the difficulty to filter out invasive plants by excluding the terms, such as “grass”, “weed” and “tree”, because many animals, such as grasshoppers, have words related to plants in their name. Thus, we decided to exclude species other than animals not in the search strings but in the paper screening in the next steps. The following are two examples of search strings in the Scopus database and Web of Science Core Collection:

Search String in Scopus (1004 records extracted)

ABS ( invasi* AND ( "ecolog* success" OR ( increas* W/3 population* ) OR ( expan* W/3 range ) OR ( success W/3 reproduc* ) ) ) AND ( LIMIT-TO ( PUBYEAR , 2021 ) OR LIMIT-TO ( PUBYEAR , 2020 ) OR LIMIT-TO ( PUBYEAR , 2019 ) ) AND ( EXCLUDE ( DOCTYPE , "re" ) ) AND ( LIMIT-TO ( LANGUAGE, "English" ) )

Search String in Web of Science (957 records extracted)

invasi* AND ("ecolog* success" OR (increas* NEAR/3 population*) OR (expan* NEAR/3 range) OR (success NEAR/3 reproduc*)); Refined by: Abstract, Pub year: 2019 - 2021, Review Articles: Excluded, Languages: English.

**Inclusion criteria for the studies:**

In both Web of Science and Scopus, we searched for suitable articles using the search strings presented in this document. Both Web of Science and Scopus allow for exclusion of the reviews and non-English publications from the results, and this option saved us time during later exclusions. We then used the online software Rayyan (https://rayyan.ai/) to perform abstract screening. The three authors evaluated all the abstracts blindly to avoid objectivity bias in papers inclusion and exclusion.

Keywords for inclusion

The invasive species was the main focus of this research. Keywords, such as “invasive”, “invasion”, “invasive species”, “non-native” and “alien”, were included to filter studies about invasive species. For the factors contributing to the ecological success of invasive species, the relevant keywords, such as “environmental factors”, “ecological success”, “population increase”, “reproductive success” and “range expansion”, were also included.

Keywords for exclusion

This research focused on only invasive animal species. Thus, studies about plants and microorganisms were removed by the keywords “plant”, “grass”, “weed”, “tree”, “algae”, “microorganism” and “fungi”. A problem with the keyword “invasive” is that it refers not only to species but also to the invasive and non-invasive methods that can be used in animal or medical experiments. Thus, we set “invasive method” and “non-invasive” as keywords for exclusion. To identify medical research that is not relevant to our interest, we used keywords such as “cell”, “cancer”, ‘’patient’’ and “disease”.

**Protocol for data collection from the full texts:**

The 179 publications included after abstract screening (see Fig. 1) were equally split and distributed to each author for full-text screening. During this step, we extracted the following information: 1) the taxa of the invasive animals, including mammals, birds, fishes, reptiles, amphibians, arthropods, molluscs and other invertebrates (e.g. earthworms, corals, planktons, starfishes); 2) the types of ecological success, i.e. the increase of population size, the range expansion or both; 3) the factors contributing to the ecological success of invasive animals, including environmental factors (climate, habitat traits, resource availability and anthropogenic factors) and biological factors (reproductive rate, physiological traits, enemy release, novel weapon, genetics, behaviour and interspecific interaction). “Habitat traits” represent factors such as habitat disturbance and land usage. “Enemy release” includes scenarios where the invasive species achieve a higher fitness compared to native species because of getting away from the parasites or diseases in their native habitat. “Novel weapon” includes cases where invasive species bring new parasites or diseases into the new habitat, which decreases the fitness of local species. All information was collected from each publication using an online survey created with Google Forms. The final results were visualized in R v4.0.3 using the ggplot2 package [10].

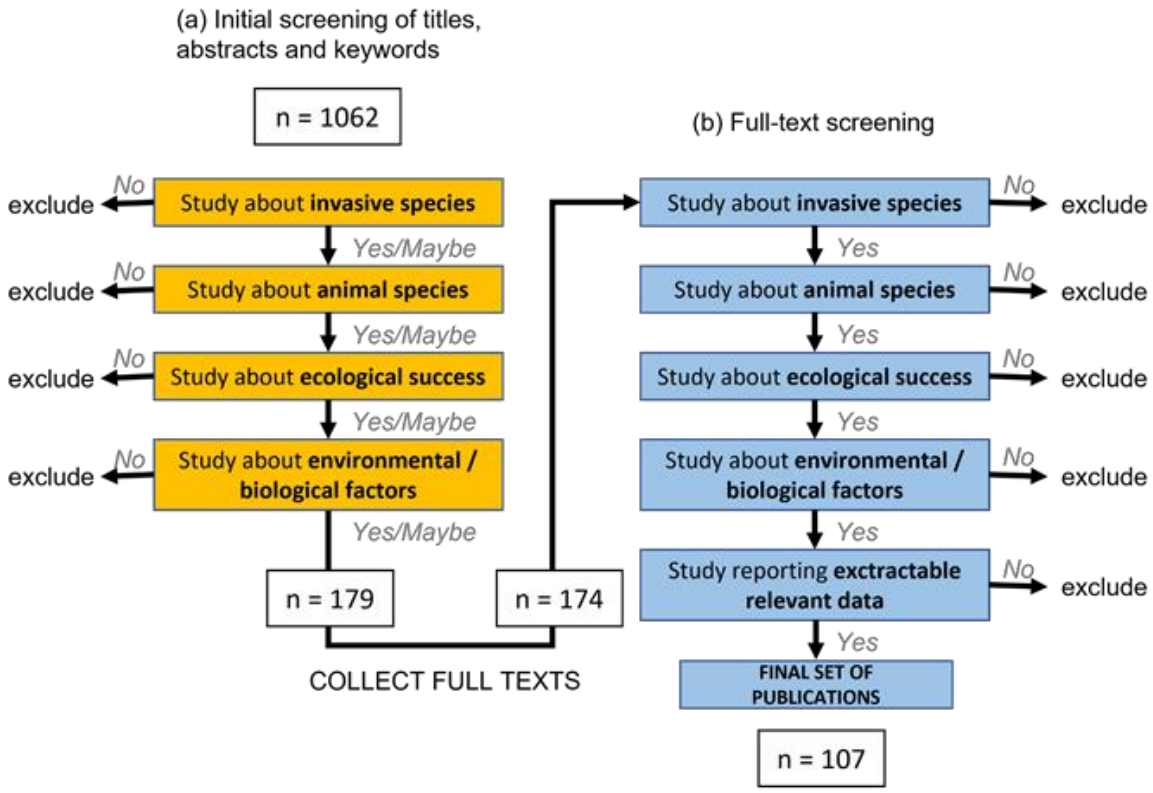


**Figure 1**. Decision tree for the filtering of our list of publications, at the abstract level (yellow boxes) and at the full-text level (blue boxes). The number of publications at each step is indicated in white boxes.

**RESULTS**

After removing the duplicates, we obtained 1062 articles with our two search strings. A total of 179 articles were included after abstract screening. However, a few documents were totally unavailable and only 174 full texts were screened in the second step. During the screening of full texts, we excluded 67 articles and we ended up with a dataset of 107 articles related to the factors contributing to the ecological success of invasive animal species (Fig. 1).

The full text screening and the information extracted from the publications reveal a clear heterogeneity of the studied taxa, the ecological success and associated factors (Fig. 2). Regardless of the ecological success feature, Arthropods are the main taxa studied, with a majority of publications on crop pests and mosquitos, all of which are closely related to humans. In terms of ecological success features, the biggest interest goes to the range expansion of alien species, either to understand their past invasion or to predict their future range. Factors explaining the invasive success of non-native animals are diverse, but the light is shed mainly on physiological traits (e.g. morphology traits, adaptation, resistance), genetics and climate. The studies on physiological traits aim at justifying how non-native species outperform native ones. The studies about genetics mainly trace the invasive history of alien species using population genetics tools. The studies about global climate change often predict the consequences of invasion or the future distribution of invasive species with the changing climate.

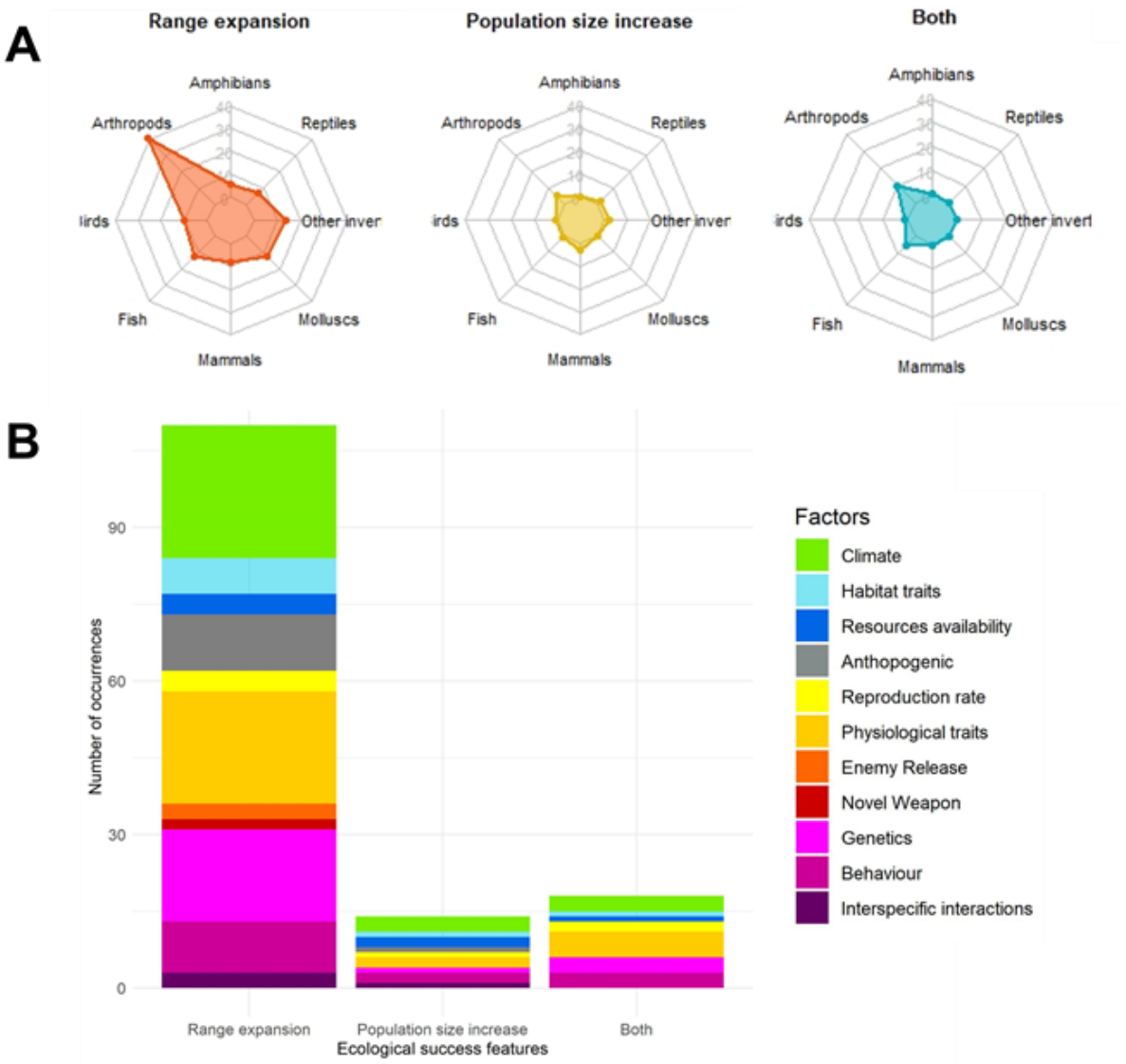


**Figure 2.** The radar charts (A) represent, for each animal taxa, the number of publications studying the increase of population size, the range expansion or both of these two ecological success features. The bar plot (B) reports the factors investigated in publications in order to understand the invasiveness of non-native animal species. Publications could measure more than one factor, increasing the number of occurrences in this graph.

## DISCUSSION

In our study, we found that arthropods are the most studied taxa in terms of the ecological success of invasive species. For each of the three categories of ecological success, range expansion of invasive species were documented in a majority of studies. Considering the factors that contribute to ecological success, three factors out of eleven were predominantly measured: climate, physiological traits and genetics.

Based on our results, we can see that research in the field of invasive animal species is mainly led by the concern about the impact of these species on human beings and societies. Some insects have been intentionally introduced in order to fight against crop pests but became themselves an issue for ecosystems, cattle or crops [5]. Some arthropods, such as tiger mosquitos, are also responsible for human health issues [11–13] due to the diseases they can carry and transmit. Thus, it is not surprising to see a majority of publications trying to understand the invasive mechanisms, to determine their current invasion level and propose control and management plans accordingly, and to foresee the future

population development of invasive arthropods. The major concern about arthropods could also explain why most studies documented range expansion as the only type of ecological success, because arthropods are often small and their distribution range is easier to monitor and more informative compared to their population size. In addition, the prediction of future distribution range under climate change could prepare local communities or countries for the potential risks and manage invasive species efficiently before they get rampant. This explains the over-representation of climatic factors and their effects on the expansion range of alien species in these publications.

The legitimate interest for human wellness may hide other problems created by invasive species. The low number of publications focusing on the impact of non-native animals on ecosystems and native species may underestimate the severity of the invasion. Direct effects of introduced species on non-productive ecosystems can have considerable effects on human beings [14], and these threats need as much investigation as current main topics in this field of research. In addition, different species can display very different invasive mechanisms and characteristics [15,16]. Generalizing the adaptiveness and invasiveness of the most studied species to the other species in the same taxa is inappropriate. We need to put more effort into our understanding of animal invasions to be truly able to anticipate and react in order to minimize their damages on both humans and biodiversity.

At the end, we would like to emphasize the objectivity of the review mapping method which allowed us to extract all relevant articles from a vast field of research in different domains (e.g. biology, ecology, computer modelling, economics). However, the scope of our systematic mapping is still narrow, due to the short time period (i.e., only the last three years) and the focus on animal species, knowing that other organisms can invade and damage new ecosystems as well. We also excluded modelling studies that only construct models based on current knowledge yet draw accurate invasion predictions, and guidelines for alien species management which give concrete tools to communities and countries. Our results only showed the heterogeneity of this research field, without much information in other animal taxa than arthropods, other features than range expansion, and other impacting factors than climate, genetics and physiological traits. To complete our overview of the recent publications dedicated to invasive species, introduced plants and their adaptiveness and invasiveness should be reviewed, as their impact on ecosystems is at least as dramatic as the impact of animal species [5,17].

**ACKNOWLEDGEMENTS**

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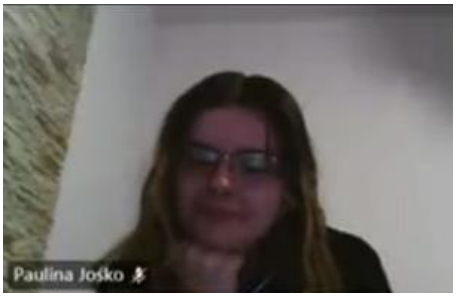
# Facing worldwide amphibian decline: A systematic review of fungal (*Batrachochytrium spp.*) infection patterns in natural populations

by

Paulina Jośko and Filip Turza




Study plan

# Facing worldwide amphibian decline: A systematic review of fungal (*Batrachochytrium spp.*) infection patterns in natural populations

Paulina Jośko, Filip Turza

## 1. Aim of study

Biodiversity loss draws high scientific interest. The number of endangered species is rapidly growing on the global scale. Among vertebrate animals, the group most vulnerable to worldwide extinction are amphibians. With around 40 % species threatened with extinction, they require special attention. Amphibians are considered to be keystone species, enabling the survival of other groups of animals. Despite the crucial importance of this group of organisms, determinants responsible for their decline are not fully understood. There are several factors considered to be the main causes of amphibian decline, such as habitat losses, climate change, invasive species, or chemical contaminants. However, the decreasing number of amphibians is noted also in relatively not transformed by human activity areas, as well as conservation zones. Emerging diseases have been included as important factor that have a potentially high impact on the amphibian decline. Fungal pathogens *Batrachochytrium* spp. pose a severe threat, as they can invoke chytridiomycosis, a lethal skin disease affecting amphibians.

In this study, we will analyse the current state of knowledge of fungal (*Batrachochytrium* spp.) infection patterns in the context of worldwide amphibian decline. First, we will provide information on the geographical distribution of wild amphibian populations, that have been tested for *Batrachochytrium* spp. Then, we will examine percentage of prevalence reported for each taxon considered, as well as sample size range, to address the potential underestimation of the data available in the literature. Moreover, we will quantify the number of studies in which developed chytridiomycosis or lethal cases were detected.

This systematic review will provide insight on the current known distributions of chytrid fungi pathogens affecting wild amphibian populations around the world. It will also reveal the extent of examination for specific taxonomic groups. Therefore, our study can identify potential gaps and arenas for more effective disease mitigation. We believe this review will be of high interest in fields of study such as herpetology, conservation biology and ecology.

## 2. Scope of the study

| **PECO** | Evidence | Keywords |
|---|---|---|
| **P**opulation | Natural populations of amphibians | (amphibia*) AND (natur* OR field OR wild*) |
| **E**xposure | Studies that measure *Batrachochytrium* spp. prevalence by sampling individuals (NOT environmental samples from habitats) | (Batrachochytrium OR Bd OR Bsal) AND (prevalence OR infect* OR occur* OR detect*) |
| **C**omparator | NA | |
| **O**utcomes | • Amphibian taxa studied<br>• Percentage of infected individuals<br>• Sample size<br>• Symptoms of chytridiomycosis or lethal cases reported | |

3. **Search-string**

Scopus:
TITLE-ABS-KEY: (amphibia*) AND (natur* OR field OR wild*) AND (Batrachochytrium OR Bd OR Bsal) AND (prevalence OR infect* OR occur* OR detect*)
N = **669** results

Web of Science:
TS: (amphibia*) AND (natur* OR field OR wild*) AND (Batrachochytrium OR Bd OR Bsal) AND (prevalence OR infect* OR occur* OR detect*)
N = **571** results

4. **Inclusion criteria for the studies**

The search in two databases generated a total number of 1240 results. Those records were initially analysed with the use of Zotero software in order to detect duplicates. Total number of records after removing duplicates was reduced to **783** results. Those records were screened for relevance according to inclusion criteria listed in the protocol presented below.

Protocol for screening records for relevance, based on title, abstract and keywords:
1. Is the study published in English? No → excluded
2. Is this publication a research article? No → excluded
3. Is the study on natural populations of amphibians? No → excluded
4. Does the study detect *Batrachochytrium* spp. (e.g.: prevalence, infection) by sampling individuals? No → excluded

All studies that fulfil above mentioned criteria will be included for subsequent full text screening. Some articles might be excluded at this stage, despite meeting all the criteria, if the full text publication will not be accessible.

5. **Protocol for data collection from the full texts**

Prior to data collecting, all full texts of the articles are going to be additionally assessed for eligibility. From all publications that qualified, we will collect following data:
1. Year of publication
2. Region (a place where research was conducted, e.g.: country)
3. Taxa (species will be categorized into groups of higher taxonomical level, e.g.: families)
4. Sample size (number of individuals will be categorized into size ranges, e.g.: <100, 100 – 500, 501 – 1000…)
5. How many individuals were infected (percent range categories, e.g.: <10 %, 10-20 %, 21 – 30 %...)
6. Whether visible signs of disease were observed (no / yes / lethal / not mentioned)

The plan of our study can be modified, if needed, according to obtained outcomes.

Report – first version

# Facing worldwide amphibian decline: A systematic review of fungal (*Batrachochytrium spp.*) infection patterns in natural populations

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**Summary**

The extinction of amphibians is one of the main concerns for herpetologists, conservation biologists and ecologists. However, there are still some uncertainties that require special attention. For example, the factors responsible for the amphibian decline are poorly understood. The *Batrachochytrium* fungi are recognized by many authors, as a major determinant leading to the global mortality of amphibians. Therefore, in this systematic review, we decided to analyze the worldwide patterns of the *Batrachochytrium* spp. infections. We focused on the year, sample size and taxa studied. Additionally, we investigated how many of the publications report the development of chytridiomycosis (i.e., visible disease symptoms or lethal cases), which is important for understanding how the fungi affect wild amphibian populations. Our results show the common prevalence of *Batrachochytrium* spp. on a global scale. However, we noted several gaps in the current state of knowledge. For instance, our results highlight which amphibian taxa remain understudied. Moreover, information about the effects of chytridiomycosis disease is insufficient. This causes difficulties in the real assessment of the effects of the fungus in many natural populations. Nevertheless, our results emphasize the need for further investigation of the impact of *Batrachochytrium* fungi on the worldwide amphibian decline.

**Keywords:** amphibians, *Batrachochytrium* spp., chytridiomycosis, prevalence

## Introduction

Biodiversity loss draws high scientific interest [1,2]. The number of endangered species is rapidly growing on the global scale. Among vertebrate animals, the group most vulnerable to worldwide extinction are amphibians. With around 40 % species threatened with extinction, they require special attention [3]. Amphibians are considered to be keystone species, enabling the survival of other groups of animals [4]. Despite the crucial importance of this group of organisms, determinants responsible for their decline are not fully understood. There are several factors considered to be the main causes of amphibian decline, such as habitat losses [5], climate change [6], invasive species [7], or chemical contaminants [8]. However, the decreasing number of amphibians is noted also in relatively not transformed by human activity areas, as well as conservation zones [9].

Emerging diseases have been included as important factor that have a potentially high impact on the amphibian decline [10,11,12,13,14]. Fungal pathogens *Batrachochytrium* spp. pose a severe threat, as they can invoke chytridiomycosis, a lethal skin disease affecting amphibians [15]. Nowadays, the studies on fungal (*Batrachochytrium* spp.) infection are rapidly growing among amphibian fauna [16,14]. Nonetheless, significant gaps in knowledge are still present, e.g., how well current data reflect the situation of amphibian decline around the world. It remains poorly understood, which amphibian taxa are tested more often than others, which regions could potentially need additional examination, or how often does the infection led to development of severe symptoms causing real losses in the amphibian populations.

In this study, we analysed the current state of knowledge of fungal (*Batrachochytrium* spp.) infection patterns in the context of worldwide amphibian decline. First, we provided information on the geographical distribution of wild amphibian populations, that have been tested for *Batrachochytrium* spp. Then, we examined prevalence of chytrid fungi reported for each taxon considered, as well as sample size, to address the potential underestimation of the data available in the literature. Moreover, we quantified the number of studies in which developed chytridiomycosis or lethal cases were detected.

This systematic review will provide insight on the current known distributions of chytrid fungi pathogens affecting wild amphibian populations around the world. It will also reveal the extent of examination for specific taxonomic groups. Therefore, our study can identify potential gaps and arenas for more effective disease mitigation. We believe this review will be of high interest in fields of study such as herpetology, conservation biology and ecology.

## Materials and methods

The study was conducted in September 2021. We used the PECO statement to define the scope of the research (Table 1). Our search focused on all studies that tested natural populations of amphibians, in which the *Batrachochytrium* spp. prevalence was measured by sampling individuals. We excluded samples obtained from habitats (e.g., eDNA from soil or water) and those taken from captive animals (trades, scientific institutions, pet shops, etc.).

**Table 1.** PECO statement used to define the scope of the research.

| **PECO** | Evidence | Keywords |
|---|---|---|
| **P**opulation | Natural populations of amphibians in which the *Batrachochytrium* spp. prevalence by sampling individuals was measured (NOT environmental samples from habitats) | (amphibia*) AND (natur* OR field OR wild*) AND (Batrachochytrium OR Bd OR Bsal) AND (prevalence OR infect* OR occur* OR detect*) |
| **E**xposure | NA | |
| **C**omparator | NA | |
| **O**utcomes | • Amphibian taxa studied<br>• Presence of infected individuals<br>• Sample size<br>• Symptoms of chytridiomycosis or lethal cases reported | |

The search was performed using Scopus and Web of Science database. Search strings in both retrieved a total of 1240 results. Those records were initially analysed with the use of Zotero software, which detected 539 duplicates. Total number of records after removing duplicates was reduced to 783 results (Figure 1). Those records were screened for relevance according to inclusion criteria based on title, abstract and keywords (see Supplementary material S1). For this purpose, we used Rayyan software [17]. To full texts analysis we included only those publications, that are research articles published in English and study natural populations of amphibians, with the focus on *Batrachochytrium* spp. detection by sampling individuals.

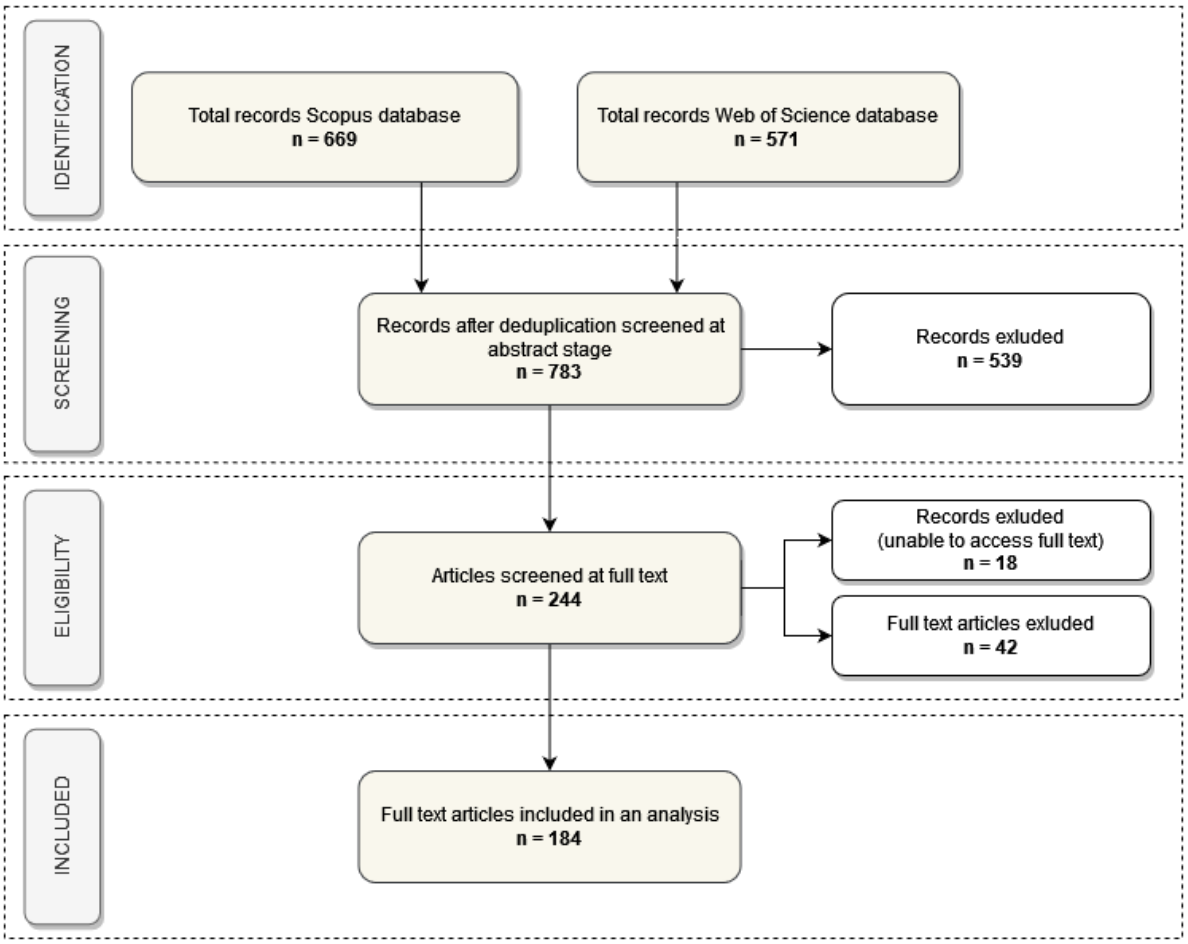


**Figure 1.** PRISMA [18] diagram presenting the workflow of our systematic review after search-string step. Values (n) are the number of publications at each stage.

All studies that fulfill the above-mentioned criteria were included for subsequent full-text screening. Some articles were excluded at this stage, despite meeting all the criteria, if the full-text publication was not accessible. All full texts of the articles were additionally assessed for eligibility, according to the same criteria as abstracts. Finally, we included 184 articles for the full text coding (Figure 1).

From all publications that qualified, we extracted following data: year of publication, region, taxa, fungus prevalence and stage of infection. With reference to taxa, we categorized species into groups of higher taxonomical level (families). In terms of region, we specified a place where research was conducted (country or state in case of the USA). Regarding sample size, we were looking for information on the total number of individuals from wild populations tested in the context of *Batrachochytrium* spp. fungal infection. Concerning fungus prevalence, we noted whether fungus

prevalence was detected or not. With regard to fungal infection, we noted whether a sign of disease such as was observed (signs of disease not observed / visible chytridiomycosis / lethal cases / not mentioned), (see supplementary material S1).

All the following analyses of the data obtained from full text coding were conducted with the use of R software [19] and following packages: tidyverse [20], ggplot2 [21], dplyr [22] and hrbrthemes [23].

**Results**

Wild amphibian populations have been tested for *Batrachochytrium* spp. prevalence on all continents where they naturally occur (Figure 2A). Out of 184 studies reviewed, 61 were conducted in North America, 27 in Middle America, 24 in Oceania, 24 in Europe, 22 in South America, 18 in Asia and only 9 in Africa (one study was counted for 2 continents). Fungi infection was detected in vast majority of papers (~91 %, Figure 2A). Only in 17 articles prevalence was not uncovered, even though the sample sizes were large, above 100 individuals (with one exception of n=16). Taxa most frequent in studies, that did not detect the fungi, was Ranidae family (~41 %). Over half of the papers focused on a single family (~59 %), however, to papers were especially exhaustive, studying 12 and 14 different families. Sample size ranged from 1 to 6830 individuals (mean value ~620, median = 258). This single individual was a first case of chytridiomycosis reported in Cuba in 2007.

Our review reveals that first research related to this topic was published for Ranidae in 2001, followed by a gap in 2002 with no publications (Figure 2B). Until 2006 number of publications was low, it did not exceed 5. There was a breakthrough in 2007, and since then frequency of articles fluctuated, but generally did not drop below 10. Highest pick was 16 publications reached in both 2013 and 2016. Data for current year are not complete, due to the time of literature search. As of September 2021, 56 families of Anura, Urodela and Apoda were studied for chytrid fungi infection. However, only 24 of those were considered in at least 5 papers. Analyzing the heatmap (Figure 2B), it is clear to see, that the focus of most researchers is on the three Anura families: Ranidae, Hylidae and Bufonidae. They have been dominating in the topic consistently since 2007, with no gap years. Overall, they have been examined in 78, 73 and 67 publications respectively. Those three taxa are followed by two Urodela families: Salamandridae and Plethodontidae with 25 and 17 mentions respectively. Other groups included in the heatmap were not studied neither as continuously, nor as thoroughly. The pattern of darker shades (Figure 2B) is very patchy for them. Therefore, the gap in the extent of study between taxa becomes evident.

Our results exposed additional issue with current state of knowledge. About 40 % of articles do not mention if there were visible symptoms of chytridiomycosis or lethal cases among sampled individuals (Figure 2C). On the other hand, a total of 60 papers state that no signs of the disease were observed. Half of them considered more than one family. Development of chytridiomycosis was discovered in as many as 47 studies, of which ~68 % were connected with additional lethal cases among sampled amphibians.

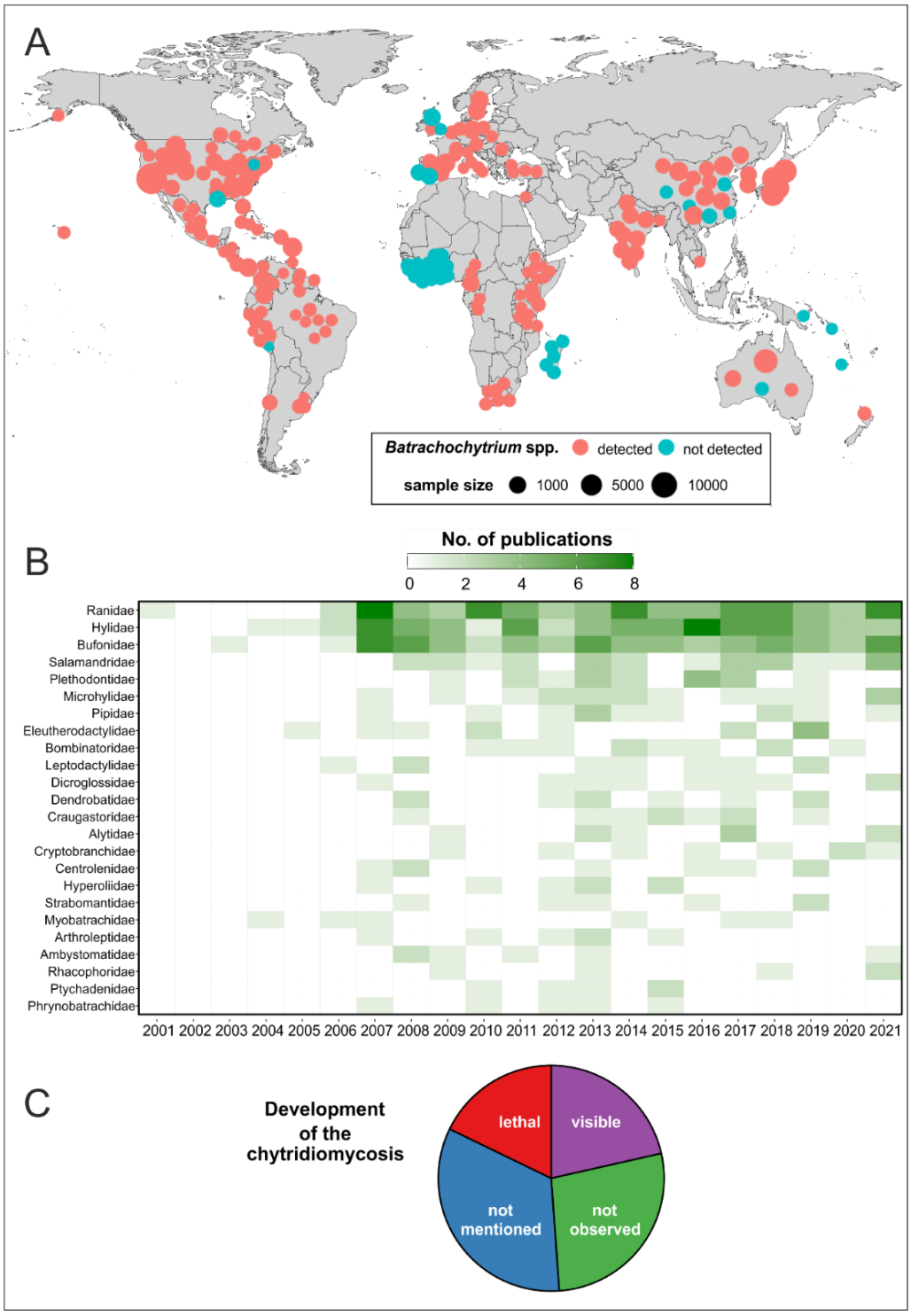


**Figure 2.** (A) Map of a worldwide distribution of a current prevalence of *Batrachochytrium* spp. in natural populations of amphibians. Number of points represents the extent of taxa studied in a given country (or state in the USA). Size of a point is scaled for the sample size (summarized for each taxon from a given region). Cases for which *Batrachochytrium* spp. was detected are marked red, while those not detected are marked blue. (B) Heatmap showing the scope of examination of fungi pathogens prevalence with the respect of taxa classification. Families, that were studied in less than 5 publications are not shown. Number of publications is presented as a color gradient, darker shades indicating more papers. (C) Pie chart depicting proportion of articles, that do not provide information on development of chytridiomycosis (not mentioned) and those that do report, whether symptoms were observed (lethal, visible, or not observed).

## Discussion

The effects of the amphibian population collapse require special research attention. Among all factors, a fungal infection caused by *Batrachochytrium* spp. is considered to be the most dangerous disease affecting the amphibian decline [24]. Here, results show the growing popularity of this issue in recent years. Moreover, our outcomes indicate the common occurrence of *Batrachochytrium* spp. among amphibian fauna and the necessity to monitor the spread of fungal infection in wild populations. Additionally, some studies point out the problem of fungus transmission by human activities. Globalization could be potentially one of the main reasons for increasing the prevalence of fungus among amphibians in recent years. Therefore, improving the biosecurity of the pathogen-free populations is necessary to inhibit the further spread of the disease around the world [25].

Our data highlight several problems with wildlife monitoring amphibians in the context of *Batrachochytrium* pathogens. Firstly, previous studies mostly focused only on a few taxa of Anura (Ranidae, Hylidae, Bufonidae) and Urodela (Slamandridae, Plethodontidae). It could be explained by the fact, that those groups have one of the highest species abundances among amphibians. Nonetheless, there are other families with exceedingly high species richness (Microhylidae, Strabomantidae, Rhacophoridae), that should not be overlooked. Secondly, over a third of the studies did not include information about visible or not observed symptoms of the chytridiomycosis disease in the studied populations. This poses a significant issue with accurate identification of species that are most vulnerable to the disease, but also those species that may potentially function as vectors of the pathogens. Clear and straightforward statement referring to visible health condition of sampled individuals paired with examination of *Batrachochytrium* spp. prevalence can help resolve this problem. Thus, the information about the stage of infection is highly important for a real assessment of the effects of the disease on wild amphibian populations. According to Berger et al. [26], several clear symptoms of chytridiomycosis disease can be distinguished, such as red skin, lethargy, abnormal body position, anorexia, and loss of reflexes. Nevertheless, it is worth noting that *Batrachochytrium* can kill individuals rapidly [27]. Therefore, the visible effects of the disease may not have been observed on the deceased individuals collected.

The geographic distribution of research also needs careful attention. To date, the largest number of studies devoted to native amphibian populations was conducted in the USA. However, in North America (NA), the number of threatened and endangered species (~440) is much smaller than, for instance, in South America (SA), where the number of threatened and endangered species is more than 2200 [28]. The studies of impact of the fungal infection on wild populations in SA is neglected in comparison, as we found nearly three times less publications than for the NA. What is more, they refer only to individual populations from the equatorial area and countries such as Ecuador, Bolivia, or Paraguay are not covered at all. Finally, future research further investigating the topic should also focus on analyzing potential factors (e.g., temperature or antifungal treatment), which may control the effects of the fungus disease in already infected populations [29,30].

To conclude, as previous authors suggest [31,32], here we also point out the evidence that prevalence of *Batrachochytium* spp. infection may be the main cause of the amphibian decline worldwide. However, we need further investigation in this field, in particular, more detailed data on the spread of the disease and susceptibility in individual taxa. Additional information on the lethal effects of the disease among amphibians, as well as potential environmental factors that may contribute to spread of the fungi are also required. This knowledge would be necessary to properly monitor the pathogen's impact on the amphibian populations and facilitate novel strategies against the spread of chytridiomycosis disease in the future [25].

**Acknowledgements**

We would like to thank the coordinator of the "Methodological Workshop in the Evolutionary Biology - practical part" course, Dr Joanna Rutkowska, and our colleagues (Junchen Deng, Pritam Dey, Agata Hołysz, Maëlle Lefeuvre, Chuchu Lu, Monika Opałek, Monika Ostap-Chęć, Aleksandra Żmuda) for great support and expert advice during online meetings.

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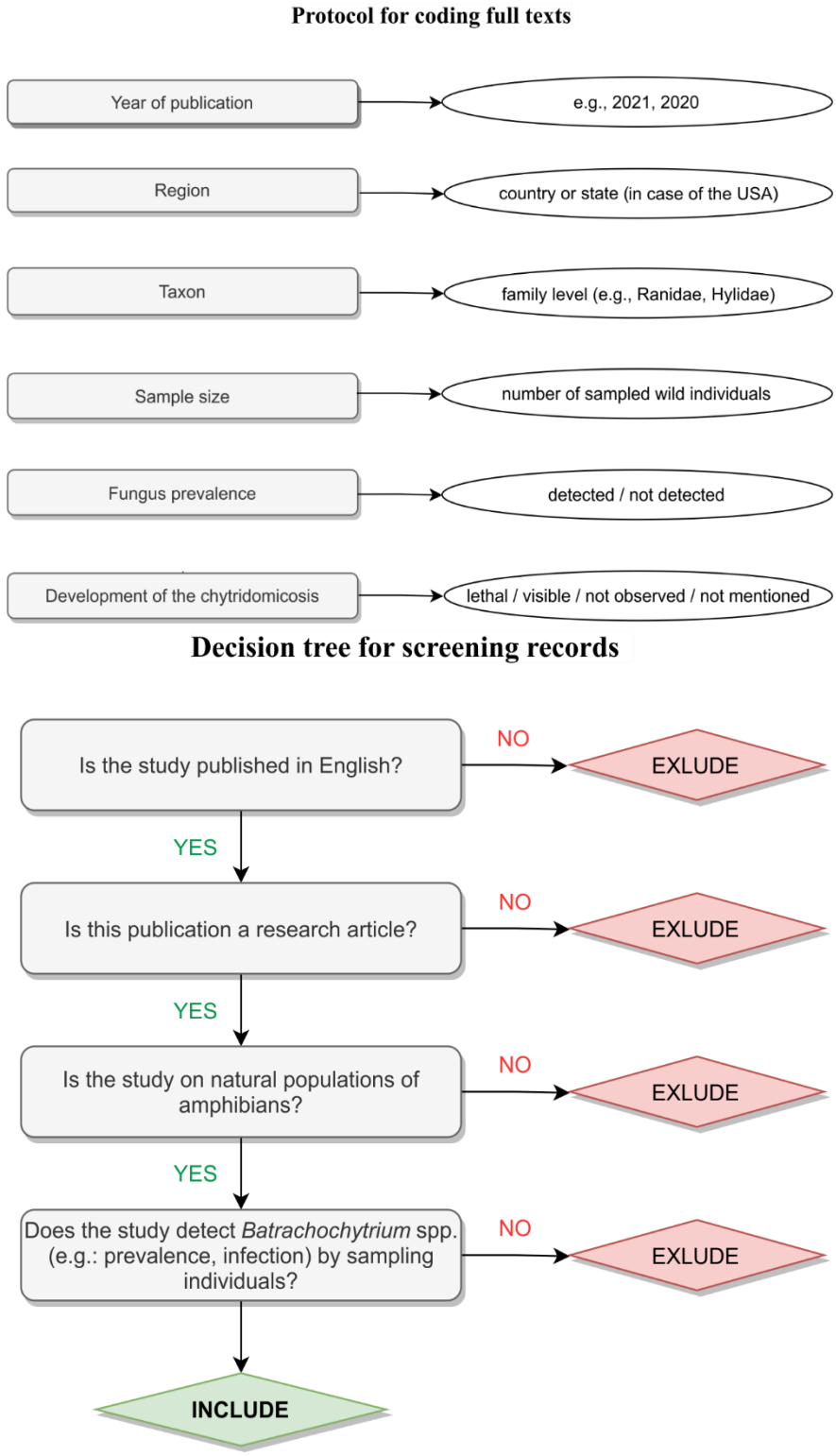
**Supplementary material**




**Supplementary figure S1.** Decision tree for screening records obtained after the literature search (top panel). Protocol was applied for both abstract and full text screening stages. Full text coding protocol (bottom panel). Some records were included in more than one category (e.g., studie conducted on several taxa or in multiple regions).

Reviews

## Reviewer: Dominika Włoch-Salamon

The authors present systematic review of fungal infection patterns in amphibians worldwide populations. From the initial 783 there were 184 articles included in the final full text coding. This allowed for answering two first out of three defined scopes of the study: *which amphibian taxa are tested more often than others, which regions could potentially need additional examination, or how often does the infection led to development of severe symptoms causing real losses in the amphibian populations.* The third question could not be answered as the authors note: *several papers do not include levels of symptoms* (which is also an interesting conclusion). I also wonder if possibly lack of infection symptoms could be connected with specificity of the fungal pathogen species, or fungal-amphibians co-adaptation, which would result in various symptoms visibility? I would appreciate if authors added some more information about *Batrachochytrium* spp.

I agree with authors, that extinction of amphibians is vast, ecologically important and clearly understudied subject. However I have doubts if the study entitles to drown conclusion: P.9.181: *To conclude, (…) the evidence that prevalence of Batrachochytium spp. infection may be the main cause of the amphibian decline worldwide.* As identifying the cause of amphibian decline was not the aim of the performed analysis. For such conclusions there should be additional factors included/excluded, such as general amphibians death/decline rate, *habitat losses [5], climate change [6], invasive species [7], or chemical contaminants [8]. (*P.2.37). Including these factors would be a substantial change of the paper scope. This is however not crucial for the presented paper in its's present form.

Meta-analysis is a well-established research tool which quantitatively combines related primary studies. As far as I can tell from my strictly theoretical knowledge about meta-analysis, the authors performed all required necessary steps, that are documented both in the text and as a figures. In my opinion meta-analysis is an extremely valuable approach. We are experiencing situation where plethora of data are gathered worldwide, using various methods, sample size etc. far from unified protocols. Not mention crisis of repeatability in science. It seems there is no a decent systematic review on *Batrachochytrium* spp. infections so far, that is why I appreciate authors idea, novelty of approach and would recommend this paper for publication J, after revision.

The authors claim that *our results highlight which amphibian taxa remain understudied* (P.1.23). However results focus only on the taxa that are present in the literature, neglecting the ones not researched, but present in geographical regions. Together with the fact that: *Families, that were studied in less than 5 publications are not shown* (in the Fig.2) (P.8.13), might give wrong impression about the level of general amphibians biodiversity. As a results readers don't know how many of the families are not included at all in the searched literature? and are excluded from the Fig.2. I think these information could be valuable, and would give the more general picture of the level of present amphibians biodiversity threatened with pathogen that need to be protected and valued.

## Reviewer: Pritam Dey

Reviewer Blind Comments to Author:
This is an excellent piece of systematic mapping that is covering one of the most concerning topics on the field of wildlife conservation. I enjoyed the reading and liked the overall representation of this work. Authors are successful to find out the diversity of the amphibian populations affected by chytrid fungi and distribution of this deadly fungus worldwide as well as identified the necessary research gaps in this topic. Moreover, there are no major mistakes found in formatting the article as per author's guidelines. I think it is worth considering the acceptance with some minor corrections and suggestions. Specific comments are as follows:

**Materials and Methods:**

Line 69: Search string is one of the most important components of systematic mapping and it gives readers a clear idea, how to search documents in different scientific databases. Not only that but also it will help readers to the replicate the study easily. Please include the search string in a way that you have used to search in different data bases.

Line 73: Inclusion and Exclusion criteria is also very important part of systematic mapping, and it is necessary to include inside the full text. Although it is presented in supplementary materials. Please include it either in words or try to combine it within the Fig 1.

Line 95: Please add the versions of the R software.

**Results**

Line 101 & 111: It is better to write single digit numbers (1-9) in words in the full text.

Line 104: Add reference for the exception case.

Line 108: Add reference.

Line 124-129: The last paragraph should be clearer with its presentation Fig 2C. I would suggest only use percentage or numbers in the text to make it more clearly understandable and add the percentages or numbers for each category that has been presented as a pie chart (Fig 2C).

**Discussion**

Line 148: It is necessary to add reference for this kind of statement. Please add the reference or references.

Line 181: Please use past for of the "suggest".

**References**

I did not notice any DOI for a single reference please go through the author's guidelines and add the DOI where it is available.

See the author's guidelines [https://royalsociety.org/journals/authors/author-guidelines/]

**Reviewer Confidential Comments to Editor:**

Although the inclusion criteria of this study only restricted in last two years of publication in the field of only research articles, the authors are successful to establish the significance of this work within the limited time that has been given. Moreover, there were not any major mistakes found in the article and considering the importance of the topic in the present time it should be encouraged to get a spotlight via successful publication. I will happily suggest this article for publication with the given corrections.

## Reviewer: Chuchu Lu

The authors carefully conducted a systematic review on the fungal infection profiles in amphibians, which mapped out several crucial factors in terms of the trends, biases, and limitations of the current research. Their results highlighted several key knowledge gaps for monitoring amphibian populations in the context of pathogen infections. This review suggest that current studies are focusing on a few particular species of high abundance with the geographical localities of the research heavily biased towards the US. The authors provided a number of recommendations for future studies including detailed suggestions and potential directions which will lead to greater understanding of this field. These recommendations will potentially help towards more applicable studies that can be contributed to the managements of the endangered species worldwide.

I enjoyed reading the manuscript which has clear aims and are supported by detailed PECO statements to explained the scope of the research. Background and the purpose of this review was well defined and is very likely to provide practical information for all researchers and general public in hopes of applying the scientific knowledge towards conservation management. Within the PECO statement, the exposure was not defined but I feel like the prevalence of the pathogen should be put in this category instead of the population section.

The screening procedures for both the abstracts and full texts were visually presented in PRISMA diagram with most of the inclusion and exclusion criteria described. However, the inclusion criteria for full text screening could be explained in more detail. Heatmap showing the number of studies across year and taxa could be replaced by more informative charts or visualization with numbers. The colours in the pie chart of figure 2 does not help to explain the problems in a constructive way. Numbers or proportions of each categories may give a more self-explanatory visualization.

The authors noted the biases of the research based on data of the geographical location the studies devoted to native amphibian populations. However, this problem can be influenced by many factors from logistic to the lack of academic facilities and research groups based in areas such as South America as they pointed out. The unproportionally number of endangered species being neglected in this field of study is concerning nonetheless. This review could have potentially included more or less criteria in the preliminary stage to determine the factors that could affect these biases.

As mentioned, the analyses of the data were carried out in R, some simple statistical analyses performed on the frequency of the studies conducted in different location, taxa, and whether the infection was reported would be good addition for a more robust interpretation of the results. Specific comments to the keywords in the search string, addition of the words “prevalence”, “infect”, “occur”, and “detect” may potentially have limited the search on Batrachochytrium studies that were relevant. Finally, the authors coded and reported the sample sizes from full text screen, however, this information was not further interpreted in the discussion.

Overall, this systematic review adds valuable information by mapping the trends and knowledge gaps in the study of fungal infection on amphibian decline. The insights and future research directions provided by the authors may be the key to more efficient and applicable outcomes acting as contributing factors for wildlife conservation.

## Reviewer: Monika Opałek

General comment:

Presented manuscript of Jośko & Turza concerns systematic analysis of research articles on fungal (*Batrachochytrium* spp.) infection patterns in natural amphibians populations. Altogether, the authors included 184 articles in the analysis. The articles were chcecked for: (i) location of samplings, (ii) amphibian taxa analysed, (iii) reported symptoms of chytridiomycosis disease, and (iv) date (year of publication). The review concerns up-to-date and important issue of worldwide amphibian decline, which, among other causes, was linked to infectious fungal disease caused by *Batrachochytrium* spp. The authors provided novel and very interesting overview of existing literature on fungal infection affecting amphibians. Importantly, they identified knowledge gaps such as: (i) understudied localities with high species richness (e.g., South America), (ii) and overlooked amphibian taxa. They also point out major limitation of multiple studies, namely the disease symptoms are often not reported.

Introduction:
I would appreciate if authors provide some more details about the chytridiomycosis disease - including information on how the disease is transferred between amphibians, what species/groups are affected, what are the symptoms, what is the estimated mortality rate, whether it is possible to cure infected animals.
Highlight research questions: consider adding some kind of points / listing in lines 46-49 e.g., "…poorly understood: (i) which amphibian […], (ii) which regions could …" and merge with previous sentence à it would immediately catch reader's attention that these are questions you want to answer within this research

Materials and methods:
Systematic review methodology follows international standards (PECO statement and PRISMA workflow) and it's correctly applied. The methods section is well written and easy to follow.

Results:
Figure 2A: Size differences of dots making sample size are relatively small and difficult to distinguish on the map, especially when they are densely aggregated. Change information of the range instead of exact numbers, e.g. "1000" à "< 1000" for the sample size. Consider choosing colours which would differ also in brightness, as for now figure printed in grey scale would be unreadable
Figure 2B: very informative and interesting - clearly shows knowledge gaps for understudied taxa

Discussion:
The authors identified and discuss important knowledge gaps that their systematic screen identified: e.g.: articles do not report how disease affect sampled animals, what is in particular importance in the context of conservation biology; there is a significant bias of the studied localities, where species-reach regions are understudied; some taxa are studied more frequently that others even if species richness is similar.
Overall, the discussion is interesting, well written and provide important insights into current knowledge and knowledge gaps about impact of *Batrachochytrium* fungi on the natural amphibian populations. Presented article can help scientist to identify most crucial areas where the new data are needed to better protect amphibians from extinction.

Minor comments:
Please remove space between numbers and % sign throughout whole manuscript
L106: "to" à "two"
L101: "counted" à "conducted in"?
L107: provide exact number with one or two decimals
L111: "5" à 5 articles per year? Or all together?
L111: "breakthrough" à what kind of breakthrough? Caused by a discovery/disease abundance?
L114: new paragraph from "As of September 2021, 56 families…"
L181: "To conclude, as previous authors suggest [31,32], here we also point out the evidence …" à "In line with previous studies/discoveries…"

Report – final version

# Facing worldwide amphibian decline: A systematic review of fungal (*Batrachochytrium spp*.) infection patterns in natural populations

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**Summary**

The extinction of amphibians is one of the main concerns for herpetologists, conservation biologists and ecologists. However, there are still some uncertainties that require special attention. For example, the factors responsible for the amphibian decline are poorly understood. The *Batrachochytrium* fungi are recognized by many authors, as a major determinant leading to the global mortality of amphibians. Therefore, in this systematic review, we decided to analyze the worldwide patterns of the *Batrachochytrium* spp. infections. We focused on the year, sample size and taxa studied. Additionally, we investigated how many of the publications report the development of chytridiomycosis (i.e., visible disease symptoms or lethal cases), which is important for understanding how the fungi affect wild amphibian populations. Our results show the common prevalence of *Batrachochytrium* spp. on a global scale. However, we noted several gaps in the current state of knowledge. For instance, our results highlight that only few amphibian taxa are extensively studied. Moreover, information about the effects of chytridiomycosis disease is insufficient. This causes difficulties in the real assessment of the effects of the fungus in many natural populations. Nevertheless, our results emphasize the need for further investigation of the impact of *Batrachochytrium* fungi on the worldwide amphibian decline.

**Keywords:** amphibians, *Batrachochytrium* spp., chytridiomycosis, prevalence

## Introduction

Biodiversity loss draws high scientific interest [1,2]. The number of endangered species is rapidly growing on the global scale. Among vertebrate animals, the group most vulnerable to worldwide extinction are amphibians. With around 40% species threatened with extinction, they require special attention [3]. Amphibians are considered to be keystone species, enabling the survival of other groups of animals [4]. Despite the crucial importance of this group of organisms, determinants responsible for their decline are not fully understood. There are several factors considered to be the main causes of amphibian decline, such as habitat losses [5], climate change [6], invasive species [7], or chemical contaminants [8]. However, the decreasing number of amphibians is noted also in relatively not transformed by human activity areas, as well as conservation zones [9].

Emerging diseases have been included as important factor that have a potentially high impact on the amphibian decline [10,11,12,13,14]. Fungal pathogens *Batrachochytrium* spp. pose a severe threat, as they can invoke chytridiomycosis, a lethal skin disease affecting amphibians. These pathogens can be spread directly by contact with an infected individual or by zoospores present in the habitat [15]. According to Berger et al. [16], several clear symptoms of chytridiomycosis disease can be distinguished, such as red skin, lethargy, abnormal body position, anorexia, and loss of reflexes. Nowadays, the studies on fungal (*Batrachochytrium* spp.) infection are rapidly growing among amphibian fauna [17,14]. Nonetheless, significant gaps in knowledge are still present, e.g., how well current data reflect the situation of amphibian decline around the world. It remains poorly understood, which amphibian taxa are tested more often than others, which regions could potentially need additional examination, or how often does the infection lead to development of severe symptoms causing real losses in the amphibian populations.

In this study, we analysed the current state of knowledge of fungal (*Batrachochytrium* spp.) infection patterns in the context of worldwide amphibian decline. First, we provided information on the geographical distribution of wild amphibian populations, that have been tested for *Batrachochytrium* spp. Second, we examined prevalence of chytrid fungi reported for each taxon considered, as well as sample size, to address the potential underestimation of the data available in the literature. Third, we quantified the number of studies in which developed chytridiomycosis or lethal cases were detected.

This systematic review will provide insight on the current known distributions of chytrid fungi pathogens affecting wild amphibian populations around the world. It will also reveal the extent of examination for specific taxonomic groups. Therefore, our study can identify potential gaps and arenas for more effective disease mitigation. We believe this review will be of high interest in fields of study such as herpetology, conservation biology and ecology.

**Materials and methods**

The study was conducted in September 2021. We used the PECO statement to define the scope of the research (Table 1). Our search focused on all studies that tested natural populations of amphibians, in which the *Batrachochytrium* spp. prevalence was measured by sampling individuals. We excluded samples obtained from habitats (e.g., eDNA from soil or water) and those taken from captive animals (trades, scientific institutions, pet shops, etc.).

**Table 1.** PECO statement used to define the scope of the research.

| **PECO** | Evidence | Keywords |
|---|---|---|
| **P**opulation | Natural populations of amphibians in which the *Batrachochytrium* spp. prevalence by sampling individuals was measured (NOT environmental samples from habitats) | (amphibia*) AND (natur* OR field OR wild*) AND (Batrachochytrium OR Bd OR Bsal) AND (prevalence OR infect* OR occur* OR detect*) |
| **E**xposure | NA | |
| **C**omparator | NA | |
| **O**utcomes | • Amphibian taxa studied<br>• Presence of infected individuals<br>• Sample size<br>• Symptoms of chytridiomycosis or lethal cases reported | |

The search was performed using Scopus and Web of Science databases. Following search string was used (presented in the Scopus format): TITLE-ABS-KEY: ((amphibia*) AND (natur* OR field OR wild*) AND (Batrachochytrium OR Bd OR Bsal) AND (prevalence OR infect* OR occur* OR detect*)). For the Web of Science, the search string was identical and we applied it in the "Topic" category (so the "TITLE-ABS-KEY" was replaced by "TS"). We retrieved a total of 1240 results from both databases. Those records were initially analysed with the use of Zotero 5.0.96.3 software, which detected 539 duplicates. Total number of records after removing duplicates was reduced to 783 results (Figure 1).

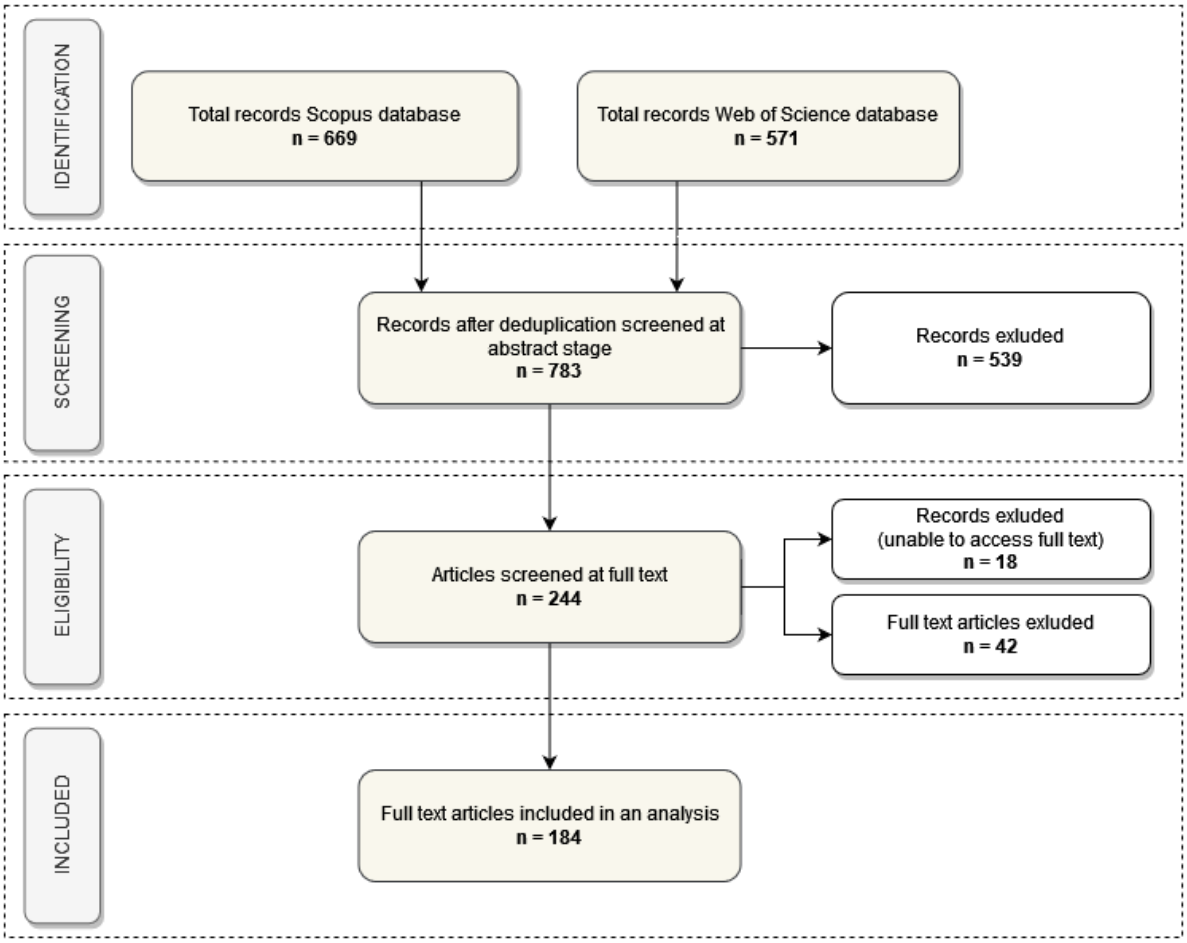


**Figure 1.** PRISMA [19] diagram presenting the workflow of our systematic review after search-string step. Values (n) are the number of publications at each stage.

Those records were screened for relevance according to inclusion criteria based on title, abstract and keywords. We included only those publications, that are research articles, published in English, study natural populations of amphibians, with the focus on *Batrachochytrium* spp. detection by sampling individuals (see Supplementary material S1). For this purpose, we used Rayyan software [18]. Studies that fulfill the above-mentioned criteria were included for subsequent full-text screening. However, some articles were excluded at this stage, despite meeting all the criteria, if the full text publication was not accessible. All full texts of the articles that we obtained were additionally assessed for eligibility, according to the same criteria as abstracts. Finally, we included 184 articles for the full text coding (Figure 1).

From all publications that qualified, we extracted following data: year of publication, region, taxa, fungus prevalence and stage of infection. With reference to taxa, we categorized species into groups of higher taxonomical level (families). In terms of region, we specified a place where research was conducted (country or state in case of the USA). Regarding sample size, we were looking for information on the total number of individuals from wild populations tested in the context of *Batrachochytrium* spp. fungal infection. Concerning fungus prevalence, we noted whether fungus prevalence was detected or not. With regard to fungal infection, we noted whether symptoms of disease were observed (signs of disease not observed / visible chytridiomycosis / lethal cases / not mentioned), (see Supplementary material S1). Thus, there were no subjective categories in our coding.

All the following analyses of the data obtained from full text coding were conducted with the use of R x64 4.1.1 software [20] and additional packages: tidyverse [21], ggplot2 [22], dplyr [23] and hrbrthemes [24].

**Results**

Wild amphibian populations have been tested for *Batrachochytrium* spp. prevalence on all continents where they naturally occur (Figure 2A). Out of 184 studies reviewed, 61 were conducted in North America, 27 in Middle America, 24 in Oceania, 24 in Europe, 22 in South America, 18 in Asia and only 9 in Africa (one study was carried out on two continents). Fungi infection was detected in vast majority of papers (~91%, Figure 2A). Only in 17 articles prevalence was not uncovered, even though the sample sizes were large, above 100 individuals (with one exception of n=16) [25]. Taxa most frequent in studies, that did not detect the fungi, was Ranidae family (~41%). Over half of the papers focused on a single family (~59%), however, two papers were especially exhaustive, studying 12 and 14 different families. Sample size ranged from 1 to 6830 individuals (mean value = 620.776, median = 258). This single individual was a first case of chytridiomycosis reported in Cuba in 2007 [26].

Nowadays, there are 74 acknowledged amphibian families. As of September 2021, we found out that 56 families of Anura, Urodela and Apoda were studied for chytrid fungi infection (see Supplementary material S2). However, only 24 of those were considered in at least five papers. Analyzing the heatmap (Figure 2B), it is clear to see, that the focus of most researchers is on the three Anura families: Ranidae, Hylidae and Bufonidae. They have been dominating in the topic consistently since 2007, with no gap years. Those three taxa are followed by two Urodela families: Salamandridae and Plethodontidae. Other groups included in the heatmap were not studied neither as continuously, nor as thoroughly. The pattern of darker shades (Figure 2B) is very patchy for them. Therefore, the gap in the extent of study between taxa becomes evident.

Our results exposed additional issue with current state of knowledge. In 33% of cases researchers did not mention if there were visible symptoms of chytridiomycosis or mortality detected among sampled individuals (Figure 2C). On the other hand, 27% state that no signs of the disease were observed. Half of them considered more than one family. Development of visible chytridiomycosis was discovered in 22% of instances, while 18% encountered lethal cases. Around 68% of reports of observed symptoms of the disease were connected with mortality among sampled individuals. Thus, they were counted twice, independently for both categories.

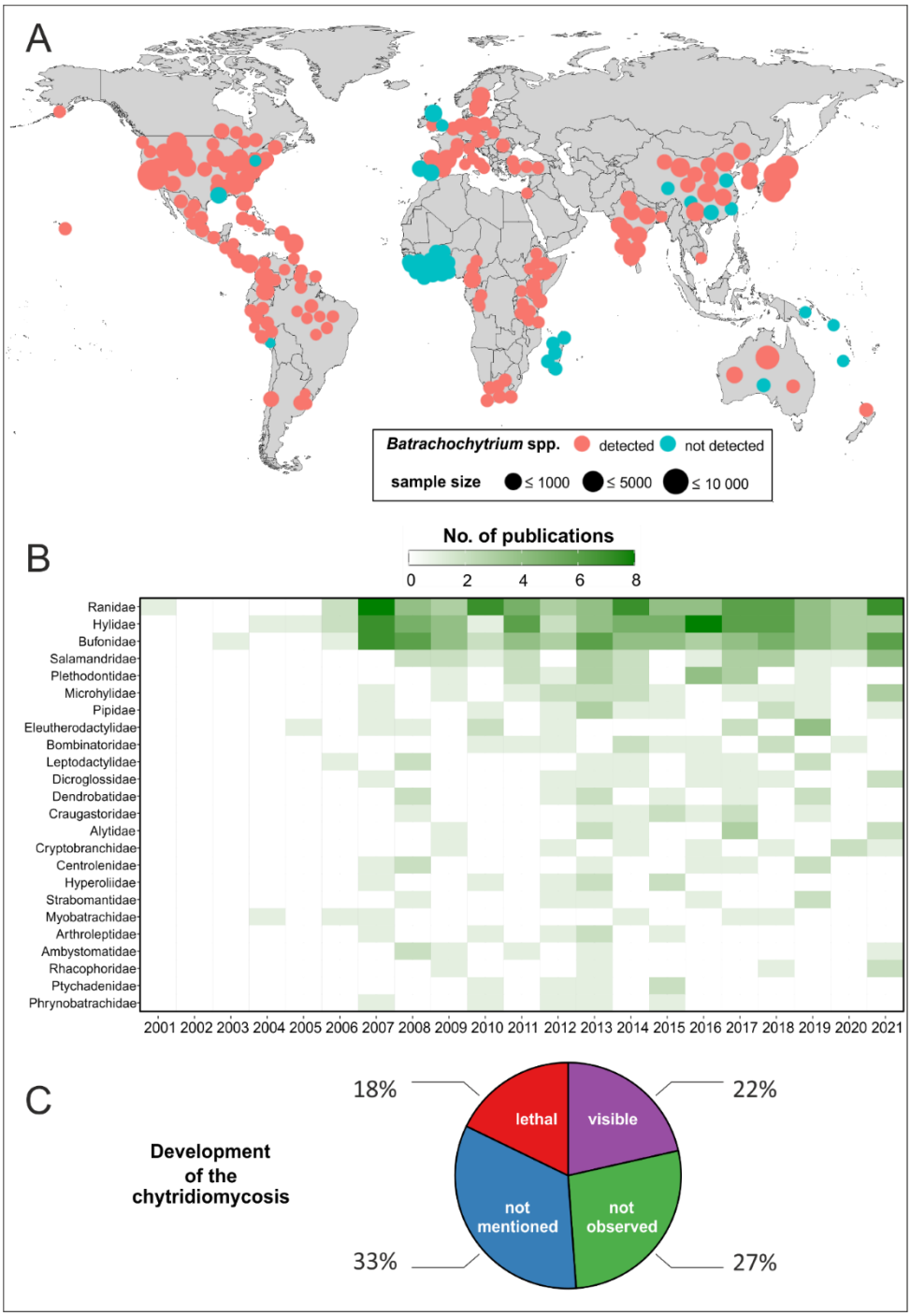


**Figure 2.** (A) Map of a worldwide distribution of a current prevalence of *Batrachochytrium* spp. in natural populations of amphibians. Number of points represents the extent of taxa studied in a given country (or state in the USA). Size of a point is scaled for the sample size (summarized for each taxon from a given region). Cases for which *Batrachochytrium* spp. was detected are marked red, while those not detected are marked blue. (B) Heatmap showing the scope of examination of fungi pathogens prevalence with the respect of taxa classification. Families, that were studied in less than five publications are not shown (for list of those taxa see Supplementary material S2). Number of publications is presented as a color gradient, darker shades indicating more papers. (C) Pie chart depicting proportion of articles, that do not provide information on development of chytridiomycosis (not mentioned) and those that do report, whether symptoms were observed (lethal, visible, or not observed). Some papers reported both visible disease and lethal cases, thus they were counted twice, independently for both categories.

**Discussion**

The effects of the amphibian population collapse require special research attention. Among all factors, a fungal infection caused by *Batrachochytrium* spp. is considered to be the most dangerous disease affecting the amphibian decline [27]. Here, results show the growing popularity of this issue in recent years. Moreover, our outcomes indicate the common occurrence of *Batrachochytrium* spp. among amphibian fauna and the necessity to monitor the spread of fungal infection in wild populations. Additionally, some studies point out the problem of fungus transmission by human activities [28]. Globalization could be potentially one of the main reasons for increasing the prevalence of fungus among amphibians in recent years. Therefore, improving the biosecurity of the pathogen-free populations is necessary to inhibit the further spread of the disease around the world [29].

Our data highlight several problems with wildlife monitoring amphibians in the context of *Batrachochytrium* pathogens. Firstly, previous studies mostly focused only on a few taxa of Anura (Ranidae, Hylidae, Bufonidae) and Urodela (Salamandridae, Plethodontidae). It could be explained by the fact, that those groups have one of the highest species abundances among amphibians. Nonetheless, there are other families with exceedingly high species richness (Microhylidae, Strabomantidae, Rhacophoridae), that should not be overlooked. Secondly, about third of the studies did not include information about visible or not observed symptoms of the chytridiomycosis disease in the studied populations. This poses a significant issue with accurate identification of species that are most vulnerable to the disease, but also those species that may potentially function as vectors of the pathogens. Clear and straightforward statement referring to visible health condition of sampled individuals paired with examination of *Batrachochytrium* spp. prevalence can help resolve this problem. Thus, the information about the stage of infection is highly important for a real assessment of the effects of the disease on wild amphibian populations. Especially, considering that *Batrachochytrium* can cause rapid mortality among individuals [30].

The geographic distribution of research also needs careful attention. To date, the largest number of studies devoted to native amphibian populations was conducted in the USA. However, in North America (NA), the number of threatened and endangered species (~440) is much smaller than, for instance, in South America (SA), where the number of threatened and endangered species is more than 2200 [31]. The studies of impact of the fungal infection on wild populations in SA is neglected in comparison, as we found nearly three times less publications than for the NA. What is more, they refer only to individual populations from the equatorial area and countries such as Ecuador, Bolivia, or Paraguay are not covered according to our review. Finally, future research further investigating the topic should also focus on analyzing potential factors (e.g., temperature or antifungal treatment), which may control the effects of the fungus disease in already infected populations [32,33].

It should be noted that this systematic review has several limitations. Firstly, it included only articles published in English. Therefore, some articles relevant to the topic, but published in other languages (e.g., from local journals) could have been overlooked. The second limitation is the use of only two search databases (Scopus and Web of Science). Although they cover a broad scale of scientific literature from the field, it is possible that additional articles could have been found with the use of additional databases (e.g., in the Science Direct repository). Moreover, as mentioned in the introduction, there are several potential determinants of the global mortality of amphibians which could be interconnected and influence one another. Thus, the third limitation is that only one factor (*Batrachochytrium* spp. infection) was the focus of this research. Finally, literature for this systematic review was obtained in September 2021, so the data for this year is going to require updating in the future.

To conclude, here we indicate the global pattern of *Batrachochytium* spp. prevalence in wild amphibian populations, confirming previous suggestions [34,35]. However, we need further investigation in this field, in particular, more detailed data on the spread of the disease and susceptibility

in individual taxa. Additional information on the lethal effects of the disease among amphibians, as well as potential environmental factors that may contribute to spread of the fungi are also required. This knowledge would be necessary to properly monitor the pathogen's impact on the amphibian populations and facilitate novel strategies against the spread of chytridiomycosis disease in the future [29].

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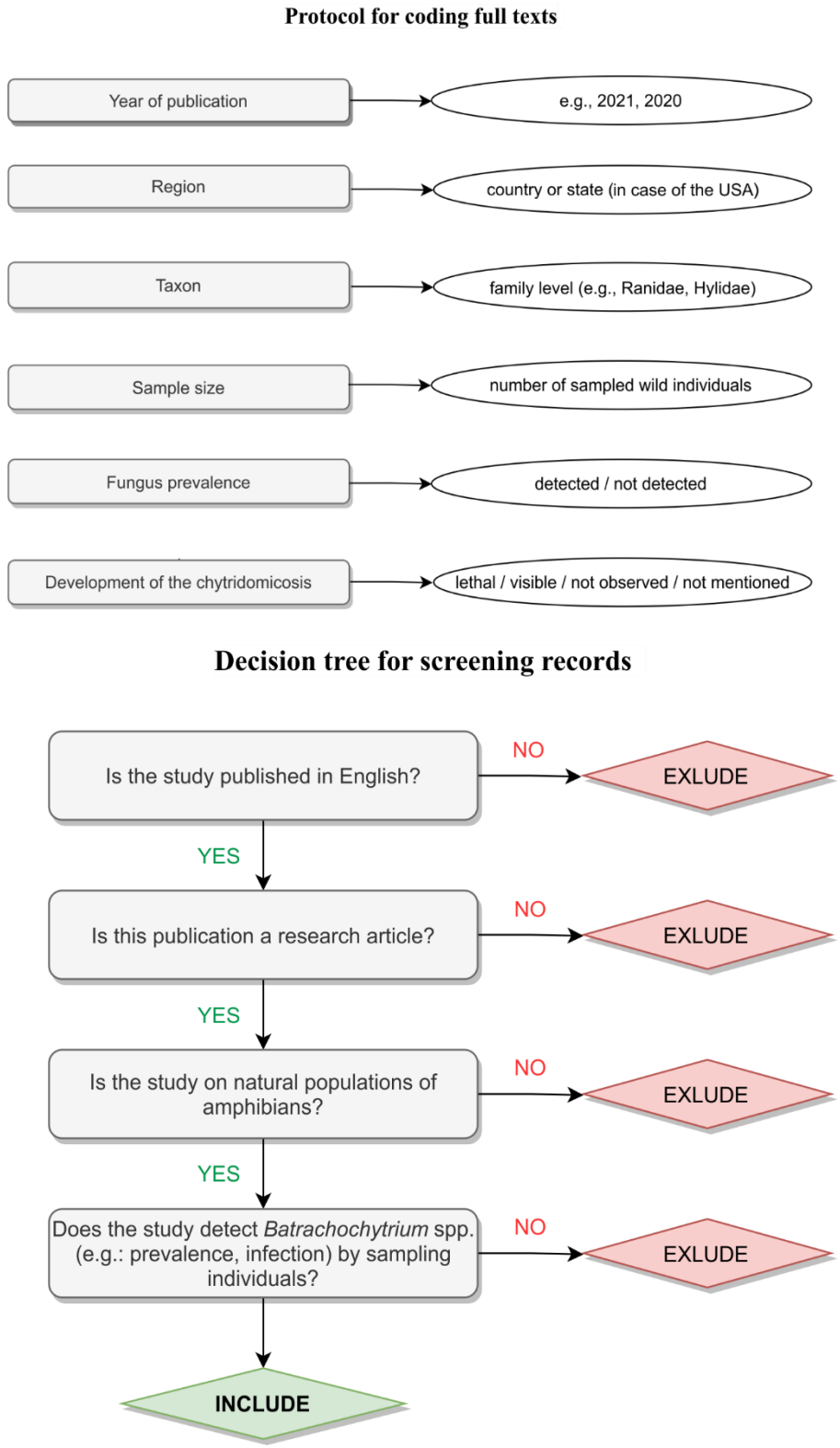
**Supplementary material**




**Supplementary figure S1.** Decision tree for screening records obtained after the literature search (top panel). Protocol was applied for both abstract and full text screening stages. Full text coding protocol (bottom panel). Some records were included in more than one category (e.g., studie conducted on several taxa or in multiple regions).

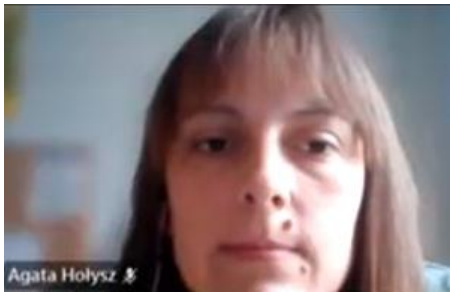
**Supplementary table S2.** List of amphibian families identified in our systematic review with number of publications that conducted study on them (N). Taxa which were examined in less than five publications in total are preseted in the middle, as other families. Additionally, list of remaining aknowledged amphibian families which were not detected in this study is included on the left.

| l.p. | Most frequent families | N | Other families | N | Not detected |
|---|---|---|---|---|---|
| 1 | Ranidae | 78 | Hynobiidae | 4 | Allophrynidae |
| 2 | Hylidae | 73 | Pelobatidae | 4 | Alsodidae |
| 3 | Bufonidae | 67 | Caeciliidae | 3 | Brachycephalidae |
| 4 | Salamandridae | 25 | Hemiphractidae | 3 | Calyptocephalellidae |
| 5 | Plethodontidae | 17 | Petropedetidae | 3 | Ceratophryidae |
| 6 | Microhylidae | 16 | Pyxicephalidae | 3 | Ceuthomantidae |
| 7 | Pipidae | 12 | Brevicipitidae | 2 | Conrauidae |
| 8 | Eleutherodactylidae | 12 | Ceratobatrachidae | 2 | Cycloramphidae |
| 9 | Bombinatoridae | 10 | Hemisotidae | 2 | Nasikabatrachidae |
| 10 | Leptodactylidae | 9 | Hylodidae | 2 | Odontobatrachidae |
| 11 | Dicroglossidae | 9 | Megophyridae | 2 | Odontophrynidae |
| 12 | Dendrobatidae | 9 | Ranixalidae | 2 | Pelodytidae |
| 13 | Craugastoridae | 9 | Rhinodermatidae | 2 | Rhinophrynidae |
| 14 | Alytidae | 9 | Amphiumidae | 1 | Sooglossidae |
| 15 | Cryptobranchidae | 8 | Ascaphidae | 1 | Rhyacotritonidae |
| 16 | Centrolenidae | 8 | Batrachylidae | 1 | Chikilidae |
| 17 | Hyperoliidae | 7 | Dermophiidae | 1 | Ichthyophiidae |
| 18 | Strabomantidae | 6 | Dicamptodontidae | 1 | Rhinatrematidae |
| 19 | Myobatrachidae | 6 | Heleophrynidae | 1 | |
| 20 | Arthroleptidae | 6 | Herpelidae | 1 | |
| 21 | Ambystomatidae | 6 | Indotyphlidae | 1 | |
| 22 | Rhacophoridae | 5 | Leiopelmatidae | 1 | |
| 23 | Ptychadenidae | 5 | Manteliidae | 1 | |
| 24 | Phrynobatrachidae | 5 | Micirixalidae | 1 | |
| 25 | | | Nyctibatrachidae | 1 | |
| 26 | | | Proteidae | 1 | |
| 27 | | | Scaphiopodidae | 1 | |
| 28 | | | Scolecomorphidae | 1 | |
| 29 | | | Siphonopidae | 1 | |
| 30 | | | Sirenidae | 1 | |
| 31 | | | Telmatobiidae | 1 | |
| 32 | | | Typhlonectidae | 1 | |

Systematic mapping of the current trends in animal personality research

by

Agata Burzawa and Chuchu Lu




Study Plan

# 1. Biases and future direction of developing experiments based on animal personality.
# 2. Mapping the research of personality traits among non-human animal taxa

Agata Burzawa, ChuChu Lu

## 1. Aim of the study

- Personality is the consistent behaviour of the individual across different times or environmental contexts. It can be examined both as a phenotypic and also a genotypic variation (Oers, 2010). Different personality traits can lead to different behavioural responses of the animal in coping with the surroundings. Describing animal personalities has evolutionary and ecological consequences (Abbey-Lee, 2018). It can be explored on two different levels (experimental and observational). Here we aimed to focus on experimental measurements. Even though there has been an increase in animal personality studies in the past decades, there are potential biases towards certain taxa. Moreover, there is still a huge knowledge gap about the factors shaping personality (Abby-Lee, 2018). It is confirmed that research on that topic provides us enormous possibilities for examining biological, genetic and environmental bases of personality perception and also in their change through different factors (Gosling, 2001).
- There are a lot of doubts around considering the nature of variation among different traits (Van Oers, 2005). Basically, categorization will also help to determine some trends among taxa as well as the different categories of personality being measured and studied.
- With rapid changes in the global climate, understanding the animal personality and how it can lead to different behavioural responses to cope with the environment will not only help in building general knowledge, but also apply further efforts in conservation and management. Some conclusions obtained on the basis of research on animal personality can be transferred later to obtaining some clues about human behaviour.
- In the systematic analysis, we want to investigate what are the global trends in animal personality. Profiling the past, current, and future direction of the non-human personality research. Determining the trends and biases in animal personality research in the last 20 years, because the popularity of this topic has doubled in written articles. We will focus on distinguishing the personality traits for different taxa and help map and shape future research direction.

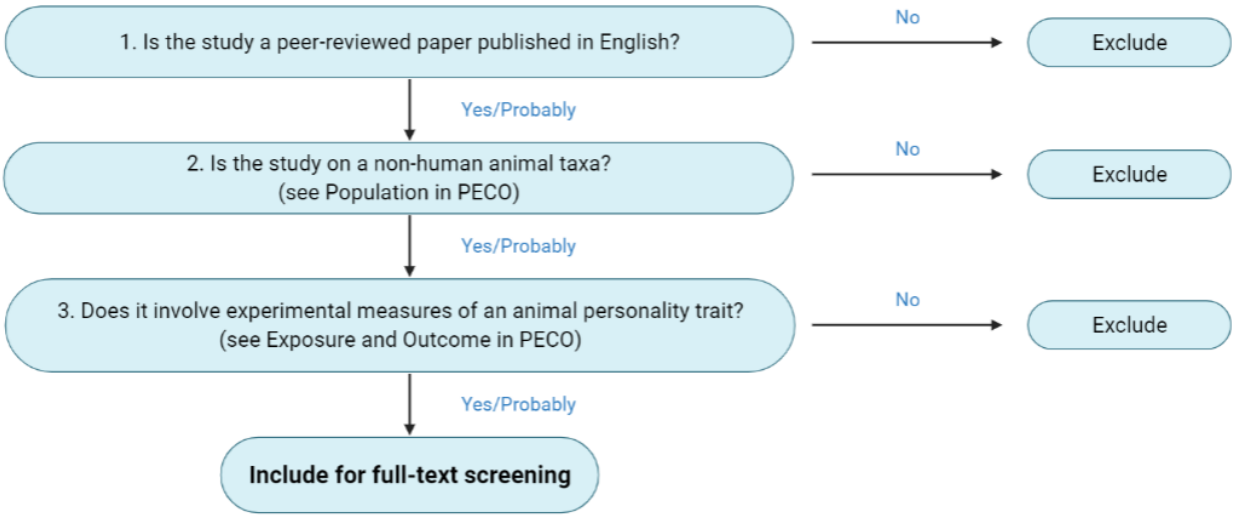
## 2. Scope of the study

- POPULATION: All animals of non-human taxa
- EXPOSURE: Experimental studies of personality traits at different life-stages
- OUTCOME: Experimental measures of personality traits

## 3. Search-string

- Keywords and filters used in Scopus and Web of Science databases on 19th September 2021. Searches were limited to journal articles and reviews in the English language.
- **Scopus**:
  - TITLE-ABS-KEY ( ( personalit* OR explora* OR aggress* OR shy* OR bold* OR "consistent behaviour*" ) W/1 ( animal* OR mammal* OR bird* OR avian* OR insect* OR reptil* OR fish* OR amphib* ) AND NOT ( human* ) ) AND PUBYEAR > 2000
- **Web of Science**:
  - TOPIC ( ( personalit* OR explora* OR aggress* OR shy* OR bold* OR "consistent behaviour*" ) NEAR/1 ( animal* OR mammal* OR bird* OR avian* OR insect* OR reptil* OR fish* OR amphib* ) NOT ( human* ) )

## 4. Inclusion criteria for the studies



**5. Protocol for data collection from the full texts**

- ❖ Copy & paste title:……
- ❖ Year: …….
- ❖ Taxon:
  - ○ Mammal
  - ○ Bird
  - ○ Insect
  - ○ Amphibian
  - ○ Reptile
  - ○ Fish
- ❖ Stage at which the measurements were obtained:
  - ● Juvenile
  - ● Adult/imago
  - ● Others
  - ● Didn't mention
- ❖ Personality trait measured
  - ○ Aggression
  - ○ Exploration/activeness
  - ○ Shyness/boldness
  - ○ Others
- ❖ Where the experiment was conducted:
  - ○ In wild
  - ○ In captivity

**Literature**:


1. Abbey-Lee R.N., Uhrig E.J., Garnham L, Lindgren K., Child S., Lovlie H., 2018, Experimental manipulation of monoamine levels alters personality in crickets, Scientific article.
2. Gosling S.D., 2001, From mice to men: what can we learn about personality from animal research?, Psychological Bulletin.
3. Oers K.V., Mueller J.C., Evolutionary genomic of animal personality, Philosophical Transaction of the Royal Society B: Biological Sciences
4. Van Oers K., De Jong G., Van Noordwijk A.J., Kempenaers B., Drent P.J., 2005, Contribution of genetics to the study of animal personalities: A review of case studies, Behaviour

Report – first version

# Shaping the future directions of animal personality research

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**Summary (maximum 200 words)**
Animal personality has been a growing discipline of empirical studies in the past decades. With many more taxa and different traits being described and tested, it is crucial to compile and map out the current trends and potential biases in the direction of the research field. The Pace-of-life theory explains how animals adapt in different environments, but very often individuals vary in their personality, which gives them different ways to cope with changes. In this systematic review, we profiled all non-human animal personality experimental studies conducted between January 2018 and September 2021. We aimed to provide a comprehensive mapping of the topic by categorizing the taxa, personality traits measured, life stages, and whether the experiments were conducted in the field or in captivity. Contrary to our expectation, we found that fish comprises the largest proportion of animal personality research. Our results showed that the majority of studies were conducted on the traits of exploratory behaviour, activeness, and the shyness and boldness continuum. Finally, there are a similar number of studies carried out in the field, captivity, or captured from the wild. Furthermore, this review found an opportunity for improvement for future experiments based on animal personality.

## Introduction

Personality is the consistent behaviour of the individual across different times or environmental contexts. It can be examined both as a phenotypic and also a genotypic variation (Oers et al, 2010). Different personality traits can lead to different behavioural responses of the animal to cope with the surroundings. Describing animal personalities has evolutionary and ecological consequences (Abbey-Lee et al, 2018). It can be explored both experimentally and through observation. Here we aimed to focus on experimental measurements. Even though there has been an increase in animal personality studies in the past decades, there are potential biases towards certain taxa. Moreover, there is still a huge knowledge gap about the factors that are shaping personality variation (Abby-Lee et al. 2018). It is confirmed that research on that topic provides us enormous possibilities for examining the biological, genetic and environmental basis of personality perception and also in their change through different influential factors (Gosling, 2001).

With rapid changes in the global climate, understanding animal personality and how it can lead to different behavioural responses to coping with the environment will not only help in building general knowledge but also apply further efforts in conservation and management. Some conclusions obtained on the basis of research on animal personality can be further applied towards the studies of anthropogenic effects. There are still debates around considering the nature of variation among different traits (Drent et al, 2005). Through the profiling and systematic mapping approach, we will help determine some trends among the taxa studied as well as the different personality traits being measured and investigated.

In this systematic review, we want to investigate the global trends in animal personality by profiling the past, current, and future directions of non-human animal research. Determining the trends and biases in animal personality research in the last four years, because the popularity of this topic has increased in written articles. We predict that there will be a greater number of studies done on primates due to their behavioural similarity to humans. We expect bias in the number of experiments conducted on adults compared to other life stages. Finally, we also expect trends or biases towards a limited number of personality traits measured in the experiments. We will focus on distinguishing the different categories of personality traits for different taxa and help map and shape future research direction.

**Material and Methods**

The scope of this study includes population: of all animals of non-human taxa, exposure: experimental studies of personality traits at different life-stages, and outcome: experimental measures of personality traits. Search term strings using Boolean logic were run through Web of Science and Scopus to collect relevant peer-reviewed literature. We subsequently narrowed to target literature published between January 2018 and September 2021 and we included the studies of all non-human animals. The following search string was used for both databases and the Scopus format is presented, TITLE-ABS-KEY ((personalit* OR explora* OR aggress* OR shy* OR bold* OR "consistent behaviour*") W/1 (animal* OR mammal* OR bird* OR avian* OR insect* OR reptil* OR fish* OR amphib* ) AND NOT ( human* ) ) AND PUBYEAR > 2018. We obtained 377 results in the Scopus database and 281 in the Web of Knowledge database. Search results were further refined to exclude non-experimental studies and studies that are irrelevant to our research question (228 results in total) (Figure 1). Study inclusion was determined objectively during an abstract screening stage carried out by two collaborators using Rayyan against a set of inclusion criteria (Figure 1), which defined pertinent population, exposure, and outcomes.

Upon inclusion of the articles in full-texts, we carried out further screening procedures by implementing a google form questionnaire. We collected data on the experimental subject (animal taxon), life stage (juvenile, larvae, adults), experimental condition (wild, captive, captured and experimented in captivity), and personality traits studied for all papers included at this stage. In results, we considered 210 obtained results after further exclusion of articles (18 articles did not meet the inclusion criteria during the full-text screening).

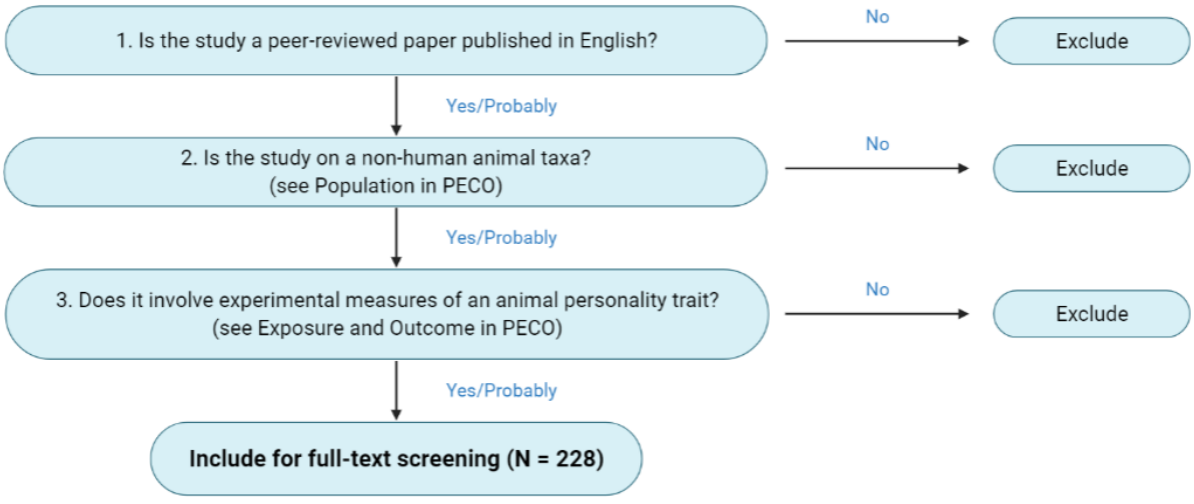


Figure 1. Illustration of the procedures carried out during the abstract screening stage by the 2 collaborators.

## Results

The number of articles on animal personality in the last four years were 2018 (N = 54), 2019 (N = 53), 2020 (N = 66), and 2021 (N = 54). There was no significant increase or decrease in the number of experimental studies since 2018.

The taxa which were the most investigated for animal personality since 2018 were fish (27,3%), mammals (23%), and birds (18,7%) (Figure 2). We suggest that measuring personality traits from these taxa is easier than from others. Most studies included in our review conducted their experiments on adult individuals (79.5%) compared to juveniles/larvae stage (14.3%) or both (8.1%). Four studies failed to define the life stage of their experimental subjects.

Categories of the personality traits measured were exploration/activeness (N = 135), shyness/boldness (N = 104), aggression (N = 33), socialbility (N = 27), risk-taking (N = 20), predatory (N = 15), neophobia (N = 9), and other categories with one study each (Figure 2).

## Discussion

Animal personalities were investigated mostly in relation to sex, body size, hormonal responses, spatial positioning, food availability, locomotion, life-history traits, responses to stress and other less abundant in studies traits (DeRango et al, 2019; Balaban et al, 2018; Anderson Bendal et al, 2018; Prasher et al, 2019). In ectotherms, personality was correlated mostly with ambient temperature. Results show that animals investigated in higher temperatures were more active, explorative and bold (Michelangeli et al. 2018). Moreover, we found plenty of variation that is supposed to be measured due to animal personality research. What taught us that not all of these variations should be compared with each other. Selection should be comprehensive and comparable (Gosling, 2001). Knowledge about different taxa investigated through animal personality sheds light on the knowledge gap which has occurred in non investigated taxa. Surprisingly, fish comprised the highest proportion of personality research in our review (Figure 2A). This is likely due to the robust methodological and apparatus development in this particular taxon (Cresci et al, 2019, Fangmeier et al, 2018, O'Neill et al, 2018). Mammals and birds are also extensively tested for their personality traits (Mammals: Brehm et al, 2020, Maiti et al, 2019. Birds: Carvalho et al, 2021, Faust and Goldstein et al, 2021), while other rest of the taxa including insects are still catching up in terms of the methodology and interests.

They have also proved that personality is changing through the lifespan (Turcsan et al, 2020). However, there were significantly more empirical studies done on subjects at the adult stage. The low percentage of experiments investigated for the long term or during all life cycles provides a future direction for animal personality experiments. Current measures of personality traits are still facing some difficulties in sometimes measuring the same personality trait with different tests, methods, and approaches (Carter et al, 2013). This was also shown in our results where multiple studies reported similar personality trait measures but the experiments were conducted in different methodology, even when the subjects were the same at the level of taxon or species. In addition, we expected far more studies of animal personality on primates due to the similarities observed in their behaviour compared to humans. However, we found little support and most mammal experiments in our results were done on various rodents. This was potentially resulted by our exclusion criteria in which we excluded humans as a keyword in our search strings. In addition, modern primate personality studies implement different personality trait measures when compared with the general animal personality experiments such as openness, conscientiousness, extraversion, and agreeableness, which are similar to human psychological measures for personality traits (Blaszczyk, 2020; Wilson et al., 2019).

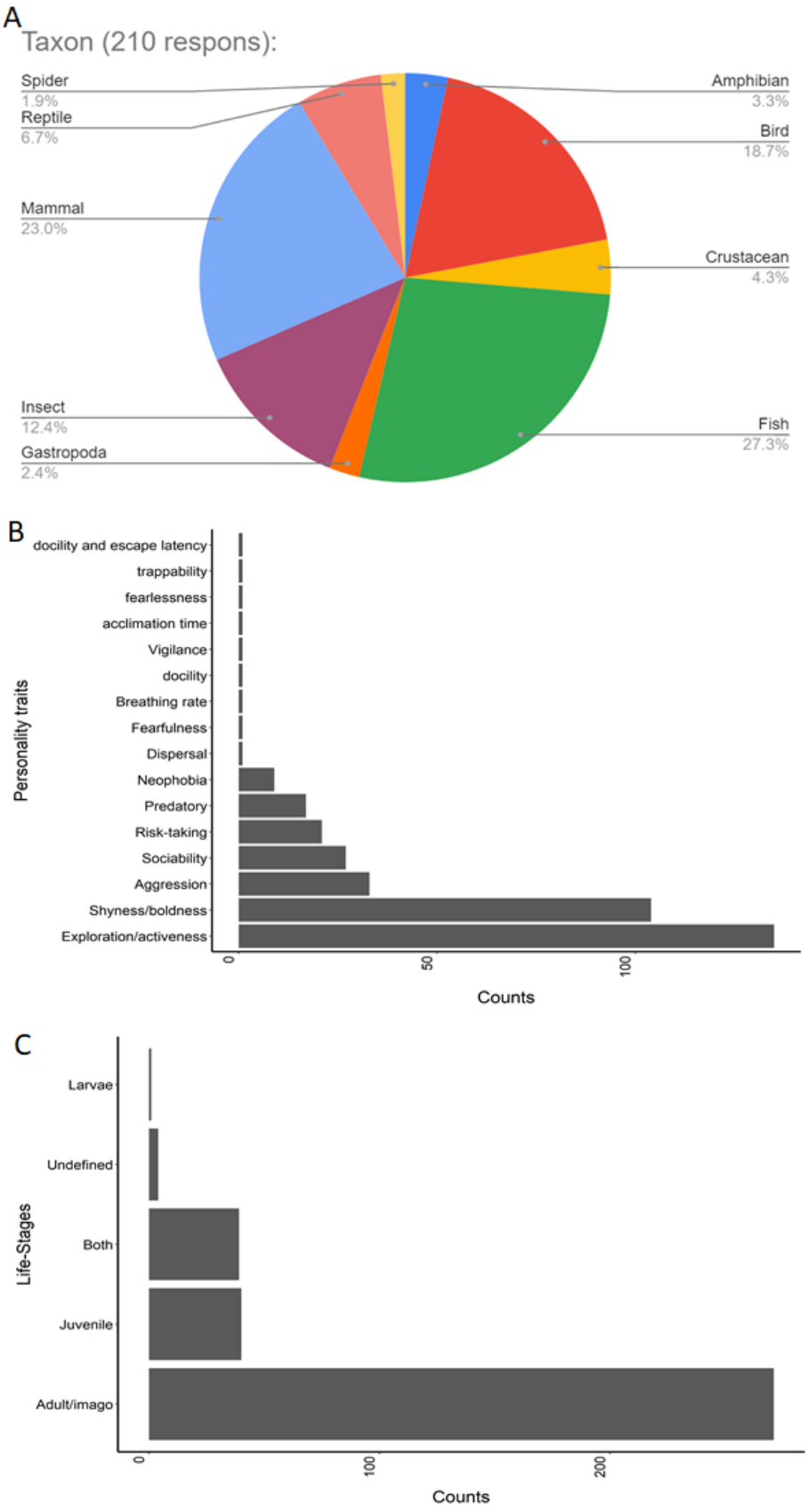


Figure 2. Bar graphs of the number of research articles conducted on animal personality. A) Proportion of studies conducted on different categories of animal taxa. B) Number of research conducted on different categories of the personality traits measure. C) Number of personality studies conducted on different life-stages.

There were no significant differences in the number of studies conducted in the field or in captivity as well as on the subjects caught from the wild. This provides a good indication that the field has been in the right direction in providing experimental studies in all conditions. However, further investigation on the approaches used in different taxa will provide greater insights into the potential knowledge and methodological gaps. We also found a field for improvement within experiments conducted in cross-species comparisons. Cross-species comparisons will not only help us fully understand what actually drives animal personality, but also will shed a light on ecological interactions between different taxa (Metha et al, 2008). Furthermore, personality has been suggested to influence the interpretation and outcome of cognitive studies (Griffin et al, 2015). Along with the growing field of animal cognition and learning, future research examining the correlations between personality and cognition can help in bringing success to both fields.

In conclusion, we found that there are some trends and biases towards particular taxa as well as the personality traits measured. The field of research is predominantly being studied on adults, which presents a significant knowledge gap in the factors and individual phenotypic plasticity in various personality traits. Future research should focus on diversifying the model organisms and also attempt to consolidate a broader standardized approach and methodology in measuring different traits across different life stages.

**Acknowledgements**
We are grateful to J. Rutkowska for helping with the development of this review and also the Jagiellonian University for providing access to the search databases.

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Reviews

## Reviewer: Hajnalka Szentgyörgyi

The manuscript presented to me is an interesting compilation of animal behaviour research. The idea of such review is timely, especially, that personality issues in animals are getting more and more attention.
The main findings of their work show a certain bias towards studies conducted on fish, however, mammals and birds were found to be also widely used, especially adult individuals. There is also a bias concerning certain personality traits (or I would rather called them behavioural) like exploratory behaviour or boldness/shyness.
The number of papers included in the analysis is impressive, but I have a certain problem with the classification of these studies. The search string the Authors used are actually filtering research not only for analysing personalities but simple behaviours like aggression, boldness etc., but lacking the search term "behavioural syndrome", which is equal to animal personality. Personality is usually described in more or less five categories: sociability, activity, novelty seeking/curiosity, problem solving/courage, stress/frustration tolerance/aggression**,** I lack most of these terms here. I think, that here lies the main weakness of their study: the lack of clarity of what are they actually analysing. Having this in mind I think it would be more correct to call their review "Shaping the future directions of animal behavioural research". Accordingly, their conclusions and also the Introduction should be changed to match the title.
Nevertheless, their conclusions are interesting and showing the research gaps in behavioural studies. Having in mind the short time the Authors had for analysing such a huge bulk of data I will not criticise the short timeframe used for their analysis, but would suggest the addition of at least one more year. An extra year can be useful for doing a correlational statistical analysis to see the trends in research in time. Not only for the number of papers published in this general topic, but also the type of research, for e.g. compare if the number of mammalian research is increasing over time. With five years there are 5 points for a statistical correlation, not much, but enough for simple answers.
This is also a weakness in their study: the conclusions are based on simple comparisons, but not on statistical analysis. I understand, that such analysis is not giving enough data for sophisticated statistical tests, but even a chi-quadrate test would be helpful to see if the difference between e.g mammal and fish studies are actually significant.
I also have some minor corrections:
All over the text: et al. is an abbreviation from Latin (*et alii* / *et aliae* / *et alia*) and therefore should be written et al., not et al,
Line 47-52: I don`t feel necessary to have these expectations in such a review. It is not an experimental study, where based on earlier result you can expect an outcome. I would leave this part out.
Line 88-89. This sentence is for the Discussion, not the Results part.
Line 92-94. Can you replace these numbers with % . The numbers are actually given in Figure 2.
Figure 2 A: Please rearrange the figure putting the slices in a size decreasing manner instead of a random manner. It would help in visualising which Taxons are on which position.
Figure 2 B: Some of these traits/behaviours can be grouped together like fearfulness and fearlessness others are somewhat unclear like trappability. I am not an expert, but this should be together with explorations or neophobia. Also why breathing rate is mentioned here? Is it a personality trait or rather a physiological one. Also dispersal seems connected with exploration to me. Or you could try to organise it according the five main personality traits if you stick with the personality context and not the behavioural one.

Line 103-105: Did you investigated this or just citing it after others? Having this database at your hand you could actually look at this data yourself and enrich your analysis.
Line 107: Is really temperature changing the personality of an ectotherm? It is rather a behavioural change due their physiology. Personality is something more stable even genetically/developmentally determined. Behaviour is something more variable and dependent on the circumstances, like temperature.
Line 119: Who have proved? If Turcsán et al., than please rephrase the sentence accordingly.
E.g.Turcsán and collegues have proven...
Line 127-128: You mention primates, but there is no mention of them in the Results. Also what do you mean by you expected far more? How many did you find?
Line 135-137: Did you exclude human bred animals e.g. dogs or horses from your analysis? They can be categorized usually neither wild nor tested in captivity, as they are raised and - especially dogs - evolved with humans.

**Reviewer: Paulina Jośko**

Tre authors conducted a systematic review of non-human animal personality research carried out in recent years. This study provides a novel, valuable contribution to the field, highlighting current trends and possible future directions. I think that the topic will be of high interest for both specialists and general readers. Overall, the manuscript was easy to read, well structured and fulfilled all the criteria required by the *Biology Letters* journal. In such short time span, authors did an extensive work in synthetising the scope of research. I would recommend this review for publication after some additional changes. Thus, I provide more detailed comments on strengths and weaknesses of the manuscript, as well as my suggestions on how this paper could be improved.

Title and abstract properly reflect subject of the systematic review. They are interesting and captivate the attention of a reader. Summary describes necessary background knowledge and study problem. Aims are clearly stated and well suited for the topic. Key results are briefly presented. However, I would like the last sentence to be more specific. Authors should state here examples of possible improvements they found, considering that the title puts an emphasis on shaping the future directions. I would also avoid using the term “Pace-of-life theory” in the abstract, especially if it is not later explained in the introduction. Such terminology may not be understandable for non-specialists and cause unnecessary confusion.

Introduction effectively explains context of the study, it’s novelty and importance. The contribution to the field of science is directly stated. The main goals were defined; however, they could be justified more thoroughly. First, authors do not comment, why they focused on experimental measurements. Moreover, the reasoning for analysing only the last four years of research is not strong enough, in my opinion. Authors claim that the discipline has been growing in past decades and that they intend to profile past directions as well. Therefore, I am not sure if reviewing papers dating back only to 2018 is adequate. I also believe that authors could further elaborate, why have they expected bias towards adults?

Scope of the study is well described in material and methods. It follows PECO structure, therefore, I suggest presenting it in a table format for easier reading. The workflow is easy to follow but could be slightly more accurate. For instance, it lacks the stage of removing duplicate records or software used to analyse and visualize the results (with proper citations). My main concern here is the search string included in the text. First, the “AND PUBYEAR > 2018” statement in Scopus limits the records to all which were published after 2018. Therefore, the year 2018 will be excluded from the search, while authors intended to review articles published since January 2018. Second, the “AND NOT

(human*)" statement can significantly limit the results obtained and I appreciate, that authors stated this in the discussion. Here I suggest the possible limiting influence of using "W/1" statement. Fixing the search for such close proximity of keywords from two categories is more restrict than using "AND".

Results are concise and comprehensible. Nonetheless, they require recalculation, as number of articles sums up to 227 in the first paragraph of this section, while authors claimed to consider 210 results. Additionally, sum of percentages of different life stage categories exceeds 100 %. The main issue is the lack of experimental condition analysis. Authors coded full texts for wild, captive, and captured conditions with reference to one of their aims, but the results for this part of the review were not described here. I suggest adding one paragraph in this section considering experimental conditions from articles included. I also think it would be practical to organise the charts in the Figure 2 according to the order of paragraphs in the results. Authors describe life stages prior to personality traits, so it would be more intuitive, to switch bar charts B and C. Finally, I do not understand why the number of personality studies conducted on Adult/Imago animals significantly exceeds 200 if the total number of studies included in the review was 210? Perhaps, it is a result of incorrectly scaled x axis?
The discussion is very well written. It sufficiently covers all the results and possible future applications of this systematic review. This section is carefully thought out and cites adequate scientific literature.

Minor comments: Authors should format references consistently, according to Biology letters guideline. They can also add correspondence e-mails and correct affiliation form, by adding superscript number. Increasing the font size around the pie chart in Figure 2A, and for labels in Figures 2B, 2C, as well as rotating the ticks on x axes 90 degrees right would improve the quality of the graphics presented.

Line 7: remove "(maximum 200 words)"
Line 33: consider changing "enormous possibilities" to e.g., "various opportunities"
Line 34: what do you mean by "personality perception"?
Line 34: consider changing "their change through different influential factors" to e.g., "their change under the influence of different factors"
Line 48: consider changing "studies done on" to e.g., "studies conducted on"
Line 58: "We subsequently narrowed" add what (results, records)?
Line 64: change "Web of Knowledge" to "Web of science"
Lines 65-66: rephrase "Search results were further refined to exclude non-experimental studies and studies that are irrelevant to our research question (228 results in total)", because now it implies that you have excluded 228 records, and from Figure 1, you have included those 228 papers
Line 74: repetition of "results", consider changing to e.g., "210 obtained records"
Line 88: be more specific with figure references (Figure 2A, Figure 2B, Figure 2C) here and on; sentence "We suggest that measuring personality traits…" can be move to discussion
Line 96: Figure 2A is a pie chart not a bar graph
Lines 107-110: It is unclear to me what variation are you referring to?
Line 116: repetition "while other rest of the…", leave either "other" or "rest"
Line 118: you start a new paragraph with "They" and it is unclear to whom it refers to?
Lines 128-129: "This was potentially resulted by" change to "This was potentially a result of"

**Reviewer: Aleksandra Żmuda**

Authors of the scientific review article carried out comparison of all non-human, experimental studies on animals personalities. As the main aim of the review they focused on making some recommendations and giving possible future trends in the researches on animal personalities basing on data received form articles published between January 2018 and September 2021. The main shows is that fishes are the most used organisms in personality studies and majority experiments is conducted on adult individuals. As authors claim using another animal taxa and concerning earlier stages of development makes big possibilities for the future development of this interdisciplinary field.
Dear authors – you did a great job despite changing the topic of your review during the curse. Congratulations!

Major comments:
- The title of the work and the written discussion are in line with the aim of study, but in abstract I found only information about already published articles. At the end is a puzzling phrase about the possibilities of improving future research, which did not satisfy my curiosity.
-I found only one mention about Pace-of-life theory in abstract. I think that elaborate on that topic in the introduction may give the reader a broader picture of the issue. Especially since the authors used 1794 words out of the 2500 available.
-While I was reading lines 36 - 43 I was a bit lost. I had been imagining that you compering personalities between individuals in the same taxa. As you wrote personality is individual trait, so it was difficult for me to think up individual way of species protection in example on insects. After a while I realised that it can based on the average species personality and differences between species. Am I correct? I will put an extra sentence in the beginning of the paragraph – something like: Although personality is an individual trait, broader knowledge about personalities ratio in a population may lead to success in species conservation.
-Methods: How did you make calculations? What software did you use to create plots?
-lines 88 – 89: Could you elaborate on that in the discussion? Why is easier to measure personality of fishes than reptails?
-I really appreciate that you put your hypotheses in the introduction part even if the results were not compatible with them. In the discussion you clarity justified opposite results on primates research.
-I could not find broader discussion on the personality traits results.
-In my opinion novelty of the study is not appropriately presented.

Minor remarks:
-line 31: which taxa are biased. Could you provide reference to that statement?
-lines 56 - 57: Did you think about putting those data into a table? It would be more legible to me.
-lines 88 - 94: I suggest to put refences to appropriate part of Figure 2 - Fig. 2A, 2B or 2C
-line 93: spelling mistake: not socialbility, but sociability
-line 96: Figure 2A-C: Text size is to small for me. It makes plots less legible
-lines 107 – 110: Those lines need some improvements, as for now it is not clear what authors write about
-line 111: I suggest: non-investigated with hyphen

**Reviewer: Maëlle Lefeuvre**

This systematic review is interesting and modern, as personality studies involve more and more animals outside primates' taxa. As explained by the authors at the beginning of their introduction, personality participates in shaping behaviours and reactions when animals face more or less sudden events in their environment. Thus, it seems self-evident that primates are not the only taxa with personality traits and studying other taxonomic groups is essential to fully understand animals' reactions and adaptation to a changing environment.

I want to congratulate authors for their impressive work and their well-organized manuscript. They collected and screened a large number of publications and covered different aspects of personality studies. Their subject was well-thought and they reported many factors to summarize the animal personality field. The taxa and personality traits are evident, but I appreciated their report of life stages and study conditions (wild, captivity) which are not the most obvious parameters of personality studies and can (and did) highlight an under-representation of some experimental conditions. Their discussion covered all the points presented in Introduction and they even reported potential limits of their work with perspicacity.

Yet, even if this subject is interesting and if this systematic review exposes knowledge gaps and gives directions for further studies in this field, I have one concern regarding the search string used by the authors. As they mentioned in their Discussion, the exclusion of the term ''human'' may have excluded relevant papers comparing non-human primates' and human personality traits. However, I am more concerned about the proximity searching operator ''W/1''. Indeed, this means that terms (or brackets) on its left and on its right must be separated by maximum one word. However, I can easily think about titles and abstracts using the expression ''aggressiveness in a group of social birds'' instead of ''social birds' aggressiveness'', or even ''boldness of wild rodents'' instead of ''wild rodents' boldness''. In those two situations, I think that the search string used by authors might have excluded relevant publications.

In addition, regarding the low number of papers excluded after abstract and full-text screening, their search string must be either impressively efficient to detect almost only relevant articles, or too narrow. I'm in favour of the second explanation, according to what I wrote earlier, and unfortunately, I think it could bias the results of this review if the 228 publications the authors included are not representative of this clearly developed field.

This was for me the main issue of this study. I have other small comments about the writing and presentation of the results.

**Figures:**

I liked the figures, they are pretty, simple and self-explanatory. I just expected more details in Figure 1. I assumed that point 3 includes not only personality traits but also the different life stages and if the animals were tested in the wild, in captivity or took from the wild to be tested. But reading the question of point 3, it is not obvious which variables you are taking into account for inclusion or exclusion of the publications.

Concerning the figure one, and especially the graph B, I wonder if it was not possible to merge some rare personality traits and combine contraries like for shyness/boldness. For instance, Fearlessness and Fearfulness could be combined, as well as the 2 studies measuring docility. And finally, the caption presents bar plots, but the graph A is a pie chart.

**Unclear parts and missing information:**

First, I would like to say that I really appreciated your statement lines 120-121 in Discussion. This is, for me, the main result fulfilling the aim of your systematic review. This is clearly a direction to follow for future studies in this field, and I appreciated to see it written in the conclusion also. I understood that the last sentence of your summary refers to this opening as well, but I would like to see it cited clearly in this last sentence.

Globally, the manuscript is clear, well-organized, well-written. There are only a few unclear paragraphs or sentences that I would like to report here to give the opportunity to the authors to improve their manuscript. The biggest incomprehension I had was the beginning of discussion, from line 102 to line 110. I don't really get the relevance of this part, especially at this stage in the discussion. I expected to read first your interpretation of your own results, and this reviewing part coming later. It could make more sense this way, with a better link between this reviewing and your results.

I also want to point our that you are reporting, in your method (lines 72-73), experimental conditions (wild, captivity and others), but this information is lacking in your aims, your scope and your results. You discuss it but I missed the results to see on which figures your discussion was based on. In addition, lines 123-126 you discuss results which seem more complex than the information you extracted from articles with your Google Form, and they are not reported in the Results either.

I was also curious about your statement in the Results part, lines 88-89, when you say that measuring personality traits in fish, mammals and birds is easier. For non-specialists of personality studies, I think it may not be cleat why it is easier. I would appreciate more explanations for this point.

**Typos and writing mistakes:**

I don't have many comments in this section, your manuscript is well-written, with a satisfactory level of English language. I would just advise to avoid exposing aims of the study in the first paragraph of introduction (line 29), it breaks the flow of the paragraph.

The sentence in lines 45-47 is unclear to me, one part may be missing. Also, just after, the last sentence in lines 51-52 could probably be written in a clearer way.

Line 64: I think you meant Web of Science

Line 118: The sentence (and paragraph) starts by ''They'', but it refers to nothing in the previous sentences, thus this sentence is unclear.

Report – final version

# Systematic mapping of the current trends in animal personality research

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**Summary**

Animal personality has been a growing discipline of empirical studies in the past decades. With many more taxa and different traits being described and tested, it is crucial to compile and map out the current trends and potential biases in the direction of the research field. In this systematic review, we profiled all non-human animal personality experimental studies conducted between January 2018 and September 2021. We aimed to provide a comprehensive mapping of the topic by categorizing the taxa, personality traits measured, life stages, and whether the experiments were conducted in the field or in captivity. Contrary to our expectation, we found that fish comprises the largest proportion of animal personality research. Our results showed that the majority of studies were conducted on the traits of exploratory behaviour, activeness, and the shyness and boldness continuum. Finally, there are a similar number of studies carried out in the field, captivity, or captured from the wild. Furthermore, this review found an opportunity for improvement for future experiments based on animal personality.

## Introduction

Personality is the consistent behaviour of the individual across different times or environmental contexts. It can be examined both as a phenotypic and also a genotypic variation (Oers et al., 2010). Different personality traits can lead to different behavioural responses of the animal to cope with the surroundings. Describing animal personalities has evolutionary and ecological consequences (Abbey-Lee et al., 2018). It can be explored both experimentally and through observation. Here we aimed to focus on experimental measurements. Even though there has been an increase in animal personality studies in the past decades, there are potential biases towards certain taxa. Moreover, there is still a huge knowledge gap about the factors that are shaping personality variation (Abby-Lee et al., 2018). It is confirmed that research on that topic provides us enormous possibilities for examining the biological, genetic and environmental basis of personality perception and also in their change through different influential factors (Gosling, 2001).

With rapid changes in the global climate, understanding animal personality and how it can lead to different behavioural responses to coping with the environment will not only help in building general knowledge but also apply further efforts in conservation and management. Some conclusions obtained on the basis of research on animal personality can be further applied towards the studies of anthropogenic effects. Although personality is an individual trait, broader knowledge about personality ratio in a population may lead to success in species conservation. There are still debates around considering the nature of variation among different traits (Drent et al., 2005). Through the profiling and systematic mapping approach, we will help determine some trends among the taxa studied as well as the different personality traits being measured and investigated.

Behavioural syndrome refers to a suite of correlated behaviours either within or between different contexts. This term is often used to analyze the correlation between personality traits. However, in this systematic review, we want to focus specifically on the empirical studies conducted on individual

personality traits. We aim to investigate the global trends in animal personality by profiling the past, current, and future directions of non-human animal research. Determining the trends and biases in animal personality research in the last four years, because the popularity of this topic has increased in written articles.

**Material and Methods section**

The scope of this study follows the structure of a PECO statement with POPULATION: all research articles on the animal personality of non-human taxa, EXPOSURE: experimental studies of personality traits at different life-stages, COMPARATOR: none, and OUTCOME: experimental measures of personality traits. Search term strings using Boolean logic were run through Web of Science and Scopus to collect relevant peer-reviewed literature. We subsequently narrowed to target literature published between January 2018 and September 2021 and we included the studies of all non-human animals. The following search string was used for both databases and the Scopus format is presented, TITLE-ABS-KEY ( ( personalit* OR explora* OR aggress* OR shy* OR bold* OR "consistent behaviour*" W/1 (animal* OR mammal* OR bird* OR avian* OR insect* OR reptil* OR fish* OR amphib* ) AND NOT ( human* ) ) AND PUBYEAR > 2018. We obtained 377 results in the Scopus database and 281 in the Web of Knowledge database. A total of 569 results were obtained after merging the duplicated articles between the databases using Zotero reference management software. Search results were further refined to exclude non-experimental studies and studies that are irrelevant to our research question (228 results in total) (Figure 1). Study inclusion was determined objectively during an abstract screening stage carried out by two collaborators using Rayyan against a set of inclusion criteria (Figure 1), which defined pertinent population, exposure, and outcomes.

Upon inclusion of the articles in full-texts, we carried out further screening procedures by implementing a google form questionnaire. We collected data on the experimental subject (animal taxon), life stage (juvenile, larvae, adults), experimental condition (wild, captive, captured and experimented in captivity), and personality traits studied for all papers included at this stage. In results, we considered 210 obtained results after further exclusion of articles (18 articles did not meet the inclusion criteria during the full-text screening). The analyses of the data obtained from full-text coding were conducted with the use of R software.

**Results**

The number of articles on animal personality obtained in the last four years were as follows, 2018 (N = 54), 2019 (N = 53), 2020 (N = 66), and 2021 (N = 54). There were no obvious trends of increase or decrease in the number of experimental studies since 2018. The taxa which were the most investigated for animal personality since 2018 were fish (27,3%), mammals (23%), and birds (18,7%) (Figure 2). Moreover, studies included in our review conducted their experiments on adult individuals (79.5%) compared to juveniles/larvae stage (14.3%) or both (8.1%). Four studies failed to define the life stage of their experimental subjects.

Categories of the personality traits measured were exploration/activeness (64%), shyness/boldness (46%), aggression (16%), sociability (13%), risk-taking (10%) predatory (7%), neophobia (4%), and other categories with one study each (Figure 2). Methods of the experimental approach were constructed with 33.8% conducted in the wild/field, 33.3% in captivity, 31.4% caught from the wild, 2.3% both, and 0.5% undefined.

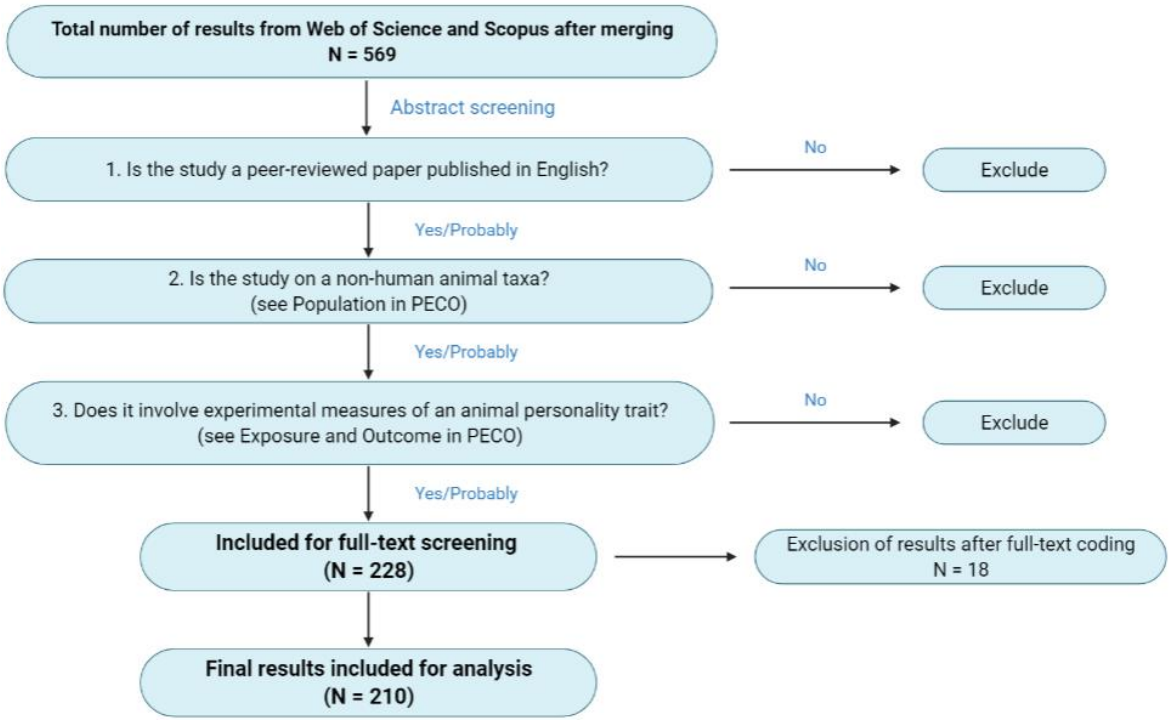


Figure 1. Illustration of the procedures carried out during the abstract screening and full text coding stage by the 2 collaborators.

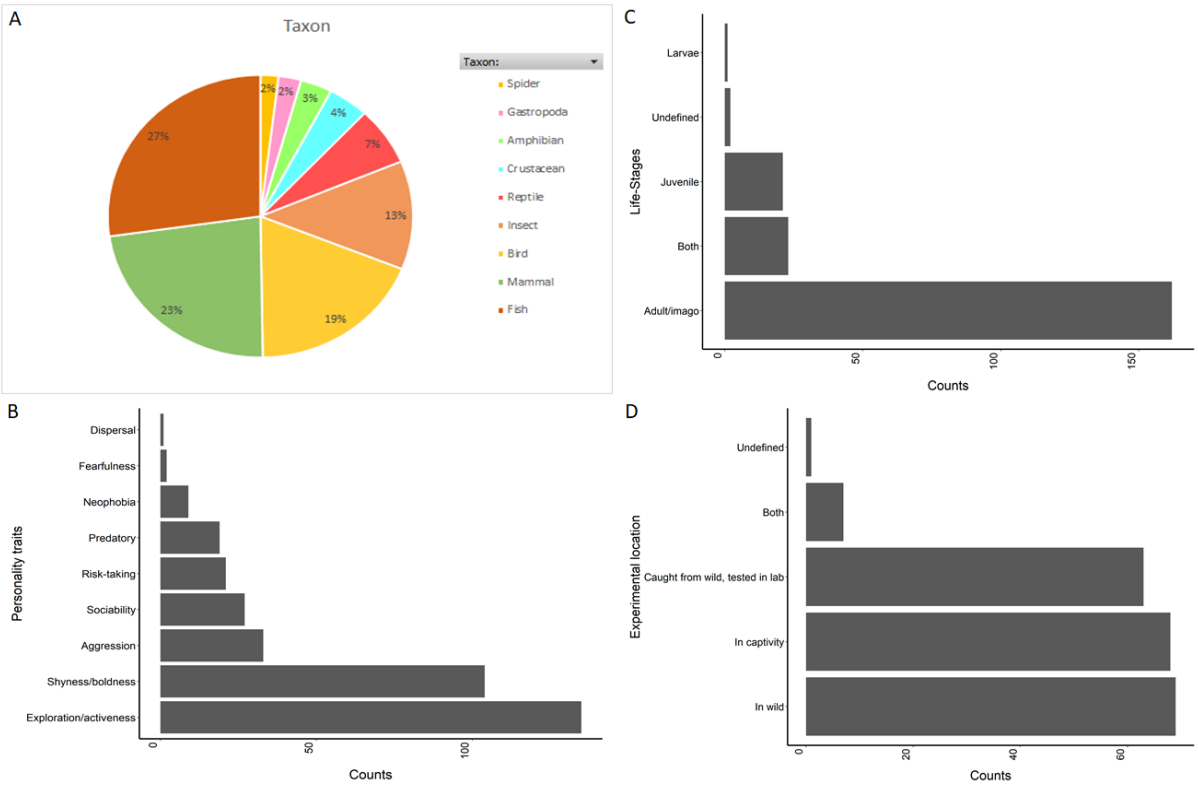


Figure 2. Bar graphs of the number of research articles conducted on animal personality. A) Proportion of studies conducted on different categories of animal taxa. B) Number of research conducted on different categories of the personality traits measure. C) Number of personality studies conducted on different life-stages. D) Number of research conducted in the wild, captivity, caught from the wild, both, or undefined.

## Discussion

Animal personalities were investigated mostly in relation to sex, body size, hormonal responses, spatial positioning, food availability, locomotion, life-history traits, responses to stress and other less abundant in studies traits (DeRango et al., 2019; Balaban et al., 2018; Anderson Bendal et al., 2018; Prasher et al., 2019). Moreover, we found plenty of variation that is supposed to be measured due to animal personality research. What taught us that not all of these variations should be compared with each other. Selection should be comprehensive and comparable (Gosling, 2001). Knowledge about different taxa investigated through animal personality sheds light on the knowledge gap which has occurred in non investigated taxa. Surprisingly, fish comprised the highest proportion of personality research in our review (Figure 2A). We suggest that measuring personality traits from these taxa is easier than from others. The first reason is that the animal personality of vertebrates is more explored. The second reason is that the ethical law allows for research on this taxa. This is likely due to the robust methodological and apparatus development in this particular taxon (Cresci et al., 2019, Fangmeier et al., 2018, O'Neill et al., 2018). Mammals and birds are also extensively tested for their personality traits (Mammals: Brehm et al., 2020, Maiti et al., 2019. Birds: Carvalho et al., 2021, Faust and Goldstein et al., 2021), while other rest of the taxa including insects are still catching up in terms of the methodology and interests.

Turcsán and colleagues have proven that personality is changing through the lifespan (Turcsan et al., 2020). However, there were more empirical studies done on subjects at the adult stage. The low percentage of experiments investigated for the long term or during all life cycles provides a future direction for animal personality experiments. Current measures of personality traits are still facing some difficulties in sometimes measuring the same personality trait with different tests, methods, and approaches (Carter et al., 2013). This was also shown in our results where multiple studies reported similar personality trait measures but the experiments were conducted in different methodology, even when the subjects were the same at the level of taxon or species. In addition, we expected many studies of animal personality on primates due to the similarities observed in their behaviour compared to humans. However, we found little support and most mammal experiments in our results were done on various rodents. This was potentially resulted by our exclusion criteria in which we excluded humans as a keyword in our search strings. In addition, modern primate personality studies implement different personality trait measures when compared with the general animal personality experiments such as openness, conscientiousness, extraversion, and agreeableness, which are similar to human psychological measures for personality traits (Blaszczyk, 2020; Wilson et al., 2019).

There were no differences in the number of studies conducted in the field or in captivity as well as on the subjects caught from the wild. This provides a good indication that the field has been in the right direction in providing experimental studies in all conditions. However, further investigation on the approaches used in different taxa will provide greater insights into the potential knowledge and methodological gaps. We also found a field for improvement within experiments conducted in cross-species comparisons. Cross-species comparisons will not only help us fully understand what actually drives animal personality, but also will shed a light on ecological interactions between different taxa (Metha et al., 2008). Furthermore, personality has been suggested to influence the interpretation and outcome of cognitive studies (Griffin et al., 2015). Along with the growing field of animal cognition and learning, future research examining the correlations between personality and cognition can help in bringing success to both fields.

There are some limitations in our systematic review. First, we did not include behavioural syndrome in our search string because we wanted to focus on experimental studies conducted to investigate individual consistent behaviours that are repeatable. Correlation between different behavioural traits was not of our interest, however, we acknowledge that there may have been some

additional research that was excluded pertaining to our narrow search criteria. Second, in our search string, we used the boolean term of W/1 which may have led to narrower results but we are also confident that we have captured the majority of experimental personality research based on the keywords selected. The future review may consider including broader terms if logistically allowed. Finally, our review was unable to capture human-bred animals that may have been studied in personality traits including dogs, cats, horses, etc. We have seen such research during the abstract screening stage but were excluded due to the methods of survey assessment and observational approach.

In conclusion, we found that there are some trends and biases towards particular taxa as well as the personality traits measured. The field of research is predominantly being studied on adults, which presents a significant knowledge gap in the factors and individual phenotypic plasticity in various personality traits. Future research should focus on diversifying the model organisms and also attempt to consolidate a broader standardized approach and methodology in measuring different traits across different life stages.

**Acknowledgements**

We are grateful to J. Rutkowska for helping with the development of this review and also the Jagiellonian University for providing access to the search databases.

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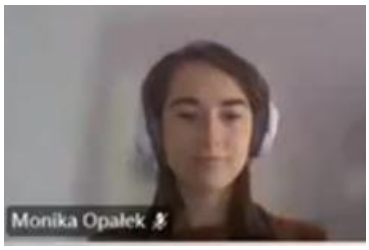
# Bee a science star – a systematic review of the primary research domains on *Apis mellifera*

by

Monika Opałek, Monika Ostap-Chęć and Aleksandra Żmuda


Study plan

# Honeybee as a model organism in science – systematic screen of publications from 2020

Monika Ostap-Chęć, Monika Opałek, Aleksandra Żmuda

## 1. Aim of study

Since ancient times, the honeybees aroused admiration and interest thanks to supplying multiple kinds of bee products. Over the centuries, people have perfected the methods of breeding honeybees and obtaining honey and wax. More recently, scientists' attention had been directed towards their advanced social structuring. Along with the development of science, honeybees had been repeatedly used as a breeding animal but also as a model organism for research on evolution of eusociality, behavior and communication in animals, as well as plant pollination.

For many years, bees have been a very important species, mainly in the field of evolution and ecology. However, in recent years, honeybees have gained much wider recognition, becoming a model species both in different biological fields, including ecotoxicology, genetics or behavioral ecology, and non-biological fields, such as programming or mathematical modeling. Thus, we aim to identify the diversity of scientific fields and the most popular ones in which bees are a research model nowadays. Such a review will not only determine the popularity of honeybee in science, but also show the potential for its use in future research.

With the progressive development, not only science fields, but also methods of working with honeybees expand and change constantly. Therefore, our goal is to check whether laboratory, field or maybe mixed experiments dominate at present, and which caste and developmental stages are studied most often. Additionally, we will note in which journals chosen articles were published. As we know, journals differ in scope of published studies, thus some of them reach for broader audience or more specialized scientist.

Within the research we plan to answer following questions:
**Question 1:** Which science fields currently use honeybee as a model organism?
**Question 2**: Are research conducted more frequently in field, laboratory or in both environments?
**Question 3**: Which honeybees' castes and developmental stages are most frequently used in research?
**Question 4**: Which journals publish research using honeybees?
Demonstrating the diversity of fields using honeybees in research will show the potential for further use of bees in new fields of science as well as in interdisciplinary projects.

## 2. Scope of the study

Tab. 1. SPIDER tool for qualitative systematic review

| | |
|---|---|
| Sample | All research articles conducted with the usage of honeybee *Apis mellifera* published in 2020 |
| Phenomena of Interest | Variety of biological fields which use honeybee as a model species |
| Design | NA (we include all research papers) |
| Evaluation | NA (we do not classify papers based on outcome) |
| Research type | Original research articles |

## 3. Search-string

We decided to include articles available in two databases: Web of Science and Scopus. Research string for each is given below:
Web of Science [Web of Science Core Collection]:
TITLE (honeybee* OR "honey bee*" OR "Apis mellifera" OR "A. mellifera") OR AUTHOR KEYWORDS (honeybee* OR "honey bee*" OR "Apis mellifera" OR "A. mellifera") AND PUBLICATION YEAR (2020) AND DOCUMENT TYPE (Articles)
Scopus:
TITLE (honeybee* OR "honey bee*" OR "Apis mellifera" OR "A. mellifera") OR KEY (honeybee* OR "honey bee*" OR "Apis mellifera" OR "A. mellifera") AND PUBYEAR (2020) AND DOCTYPE ("ar")
We decided to exclude abstracts screening from our search due to the fact that multiple papers use honeybees as comparison or mention them as a model example, even if the research does not concern honeybees. Simultaneously, when the research is conducted on honeybees, one of the names used in search string is always used in the title or keywords.

## 4. Inclusion criteria for the studies

As we are interested only in newest available data, only original scientific articles published in 2020 were considered, without review articles or book chapters. We also assume that collection of articles published in 2020 is representative for whole set of research using honeybee. We exclude grey literature, to ensure we were searching through fully set, reviewed, original and precisely methodical described studies. In addition, non-English articles have been excluded.

Tab. 2. Protocol for screening the abstract for eligibility

| Question | If answer is NO then exclude [E] or include [I] |
|---|---|
| 1. Was the article published in 2020? | E |
| 2. Was the article written in English? | E |
| 3. Is it a research article? (excluding reviews, letters, book chapters, data sets, only abstracts, etc.) | E |
| 4. Is the honeybee *Apis mellifera the* main focus of the study?<br>Excluding articles concerning:<br>A) biology of their pest/pathogens/predators,<br>B) application or parameters of bees' products (pollen, venom, royal jelly, wax, cerumen, propolis, bee bread, honey)<br>C) biodiversity of bees or environments<br>D) different species from genus *Apis* i.e. *A.cerana*<br>E) profitability of beekeeping, new trend, machines in beekeeping | E |

## 5. Protocol for data collection from the full texts

**Research fields**

10 categories of research fields were established based on our own experience and knowledge, as well as Web of Science database categories (Table 3). Some of the articles extracted for systematic review can be assigned to more than one category. We also note that if during the review of the articles, there will be studies from a field other than the one originally specified, they will be added as another category.

Tab. 3. Categories of research fields

| Category | Specifications |
|---|---|
| Systematics | subspecies, taxonomy |
| Anatomy and microbiology | body parts, organs, gut microbiota, symbionts, glands morphology |
| Behavior | learning, memory, addictions, communication |
| Toxicology | pesticides, heavy metals, air pollutants, aromatic hydrocarbons, insecticides |
| Physiology | endocrinology, immunology, organs functioning, reproduction, growth and development |
| Ecosystem services | pollination, different environments |
| Evolution | evolution of eusociality, division of labor, inheritance |
| Beekeeping | veterinary, disease treatments, breeding |
| Informatics, robotics, computer science, mathematics | Honeybee Search Algorithm |
| Other | All others which do not match to categories mentioned above |

**Experimental environment:**

For each article we will define the research environment: **field, laboratory or mixed.** If during the study, the entire experimental part was performed under natural conditions in the field, the study will be classified as field study. Similarly, if all experimental treatments are performed under controlled conditions in a laboratory, the tests will be classified as laboratory studies. It is worth noting that this classification will be applied to the experimental part of the research. For example, if all treatments are performed in the field and then the bee samples are analyzed in the laboratory for molecular or biochemistry, these studies will still be qualified as field studies. In case of part of the experiment being performed in the field and part in the laboratory, the research will be classified as mixed.

**Journals**

We will also note the information about the journals in which chosen papers were published.

**Casts and development stages:**

The chosen articles will be grouped according to the honeybees' caste (workers, drones or queens) and their stage of development (eggs, larvae, pupa, adult) used in the experiments. Studies can be classified to multiple categories at the same time if more than one caste or developmental stage is used.

Report – first version

# Bee a science star – a systematic screen of publications from 2020

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Summary

Honeybee is a widely used model organism. Traditionally, research on honeybees had been focused on pollination, evolution of eusociality and beekeeping. Nowadays, researchers in variety of biological and non-biological fields design experiments with a usage of honeybee. Here, we aimed to systematically analyse research articles published in 2020 to answer following questions: [Q1] which science fields currently use honeybee as a model organism, [Q2] are research conducted more frequently in field, laboratory, in both environments, or only via computer modelling, [Q3] which honeybees' castes are most frequently used in research, and [Q4] which journals publish research using honeybees.

Extracted publications most frequently concerned physiological and behavioural fields, however majority of studies were classified to more than one category. Therefore, we analysed how articles could be grouped and concluded that research most often combine systematics, genetics, anatomy and evolution or toxicology, physiology and behaviour. As expected, honeybees' workers are used in majority of experiments and most of research are conducted in laboratory environments. In total, analysed articles were published in 202 journals with broad range of specialisation.

## 1. Introduction

Since ancient times, the honeybees aroused admiration and interest thanks to supplying multiple kinds of bee products. The natural honey is claimed to be the first sweetener available for *Homo sapiens*, harvested as early as in the Stone Age [1]. For centuries, products such as honey, royal jelly or venom have been widely used both as food and medicines. Consequently, honeybee had been domesticated approximately 7000-10000 years ago [2]. Since then, people have perfected the beekeeping methods and developed various applications for their products [3].

Along with the development of science, honeybees had been repeatedly used not only as a breeding animal but also as a model organism in research. Scientists' attention had been directed towards their advanced social structuring and led to development of research on e.g., evolution of eusociality [4, 5], behavior and communication in animals [6-8] or plant pollination [9]. More recently, honeybee is gaining much wider recognition is science, becoming a model species in variety of biological and non-biological fields. However, how widely the honeybee is used, has not been analyzed yet.

The extensi
ve use of bees has forced scientists to develop standard research methods [10-12], what in turn enabled transfer of many experiments from the field to laboratories. The rapid progress of science generates so much data, that presumably research on bees can be performed without using bees, but only based on available datasets and results.

An important aspect of research on honeybees is also - where such results are published. Journals are characterized by a different degree of specialisation and range. Publications in most prestigious international journals with high impact factor, require not only highest research quality and broad scientific importance of discoveries, but often chose only ‘catchy’ research. Moreover, journals differ in scope of published studies, thus some of them reach for broader audience or more specialized scientist.

The aim of our study was to follow scientific publications from 2020 in which the honeybee was the research model and analyze these studies in terms of the field of science, type of research, analyzed bee caste, and the journal in which it was published. This systematic review allowed us to answer the following questions: [Q1] which science fields currently use honeybee as a model organism, [Q2] are research conducted more frequently in field, laboratory, in both environments, or only via computer modelling, [Q3] which honeybees’ castes are most frequently used in research, and [Q4] which journals publish research using honeybees.

## 2. Materials and Methods

Classification of articles for systematic review was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Supp.1). Initially, we established SPIDER statement to support our research string.

| | |
|---|---|
| **S**ample: | All research articles conducted with the usage of honeybee *Apis mellifera* |
| **P**henomena of **I**nterest: | Variety of biological fields which use honeybee as a model species |
| **D**esign: | NA (we include all research papers) |
| **E**valuation: | NA (we do not classify papers based on outcome) |
| **R**esearch type: | Original research articles |

Then, the search strings were run through two science databases:

1) Web of Science:

TITLE(honeybee*OR“honey bee*”OR“Apis mellifera”OR“A. melifera”)OR AUTHOR KEYWORDS(honeybee*OR“honey bee*”OR“Apis mellifera”OR“A. melifera”)AND PUBLICATION YEAR(2020)AND DOCUMENT TYPE(Articles)

2) Scopus:

(TITLE(honeybee*OR"honey bee*"OR"Apis mellifera"OR"A. melifera")OR KEY(honeybee*OR "honey bee*"OR"Apis mellifera"OR"A. melifera"))AND(LIMIT-TO( PUBYEAR,2020)) AND(LIMIT-TO(DOCTYPE ,"ar"))

Abstracts were excluded from search string, since multiple papers use honeybees as comparison or mention them as an example, even if the research does not concern honeybees. Simultaneously, when the research is conducted on honeybees, one of the names used in search string is always used in the title or keywords.

2.1. Abstract screening

Next, we deduplicated extracted articles and conducted abstract screening in Rayyan [13]. All authors performed independent abstract screening of all articles using the protocol (Tab.1). As we were interested only in newest data, only original scientific articles published in 2020 and written in English were considered. We assumed that such collection of articles is representative for whole set of research using honeybee. We excluded review articles, book chapters, grey literature and articles where honeybee wasn't main focus of the study (see Tab.1, Question 4).

Tab. 1. Protocol for screening the abstract for eligibility

| Question | If answer is NO then exclude [E] or include [I] |
|---|---|
| 1. Was the article published in 2020? | E |
| 2. Was the article written in English? | E |
| 3. Is it a research article? (excluding reviews, letters, book chapters, data sets, only abstracts, etc.) | E |
| 4. Is the honeybee *Apis mellifera the* main focus of the study?<br><br>Excluding articles concerning:<br>A) biology of their pest/pathogens/predators,<br>B) application or parameters of bees' products (pollen, venom, royal jelly, wax, cerumen, propolis, bee bread, honey),<br>C) biodiversity of bees or environments<br>D) different species from genus *Apis* i.e. *A.cerana*,<br>E) profitability of beekeeping, new trend, machines in beekeeping, veterinary, disease treatments, breeding, artificial nutrition | E |

2.2. Full text screening

The articles qualified for full text screening were randomly divided between all authors, where one article was screened by one person. Four aspects of each article were analyzed (i) research field, (ii) experimental environment, (iii) honeybees' cast and (iv) journal in which the research was published. Ten categories of research fields were established (Tab.2). Categorization of articles was not exclusive, meaning that one publication could be assigned to more the one research field.

Categorization of experimental environment was exclusive, meaning that one article was assigned to one category: research conducted in (i) field, (ii) laboratory, or (iii) both in field and laboratory experiments. Research concerned only about mathematical modeling or simulations were assigned to category (iv) computer modeling. The classification was applied to the experimental part of the research. In case of all treatments being performed in the field, followed by sample analysis conducted in laboratory, the article was still qualified as field study.

The articles were also grouped according to the honeybees' caste (workers, drones or queens) used in the experiments. Studies could be classified to multiple categories at the same time.

Tab. 2. Categories of research fields

| Category | Thematic scope |
|---|---|
| Anatomy | body parts, organs, gut microbiota, symbionts, glands morphology |
| Behavior | learning, memory, addictions, communication |
| Ecosystem services | pollination, different environments, conservation biology |
| Evolution | evolution of eusociality, division of labor, inheritance |
| Genetics | DNA and RNA sequencing, mitochondrial DNA, microsatellites, |
| Informatics | non-biological, computer-based research, algorithms, simulations, robotics, mathematic |
| Methodological | comparison of efficiency of methods, protocols |
| Physiology | endocrinology, immunology, organs functioning, reproduction, growth and development |
| Systematics | subspecies, taxonomy |
| Toxicology | pesticides, heavy metals, air pollutants, aromatic hydrocarbons, insecticides |

### 2.3 Canonical Correspondence Analysis

We performed Canonical Correspondence Analysis (CCA) in Past 3 software to analyse relationships between three types of collected data: journal, science fields assigned to articles (group factor) and experimental environments of studies (environment variable).

## 3. Results

Research fields: Most frequently the articles were assigned to category physiology (253 articles) and behaviour (192). More than 100 articles were also concerned about genetics (134), toxicology (124) and anatomy (109). Ecosystem services were referred in 69 articles, then least frequently articles were involving systematics (43) and evolution (38). There were 35 methodological articles and 31 non-biological (including informatics, robotics, computer science and mathematics) (Fig.1A).

Honeybees' cast: The vast majority of research are conducted on honeybee workers (440 articles, 92%), while queens were used in 50 articles (10,5%) and drones only in 32 (6,7%) (Fig.1A)

Experimental environment: More than half of analysed research included experiments conducted only in laboratory (283 articles, 59,7%). One fifth of experiments were conducted in field (94 articles, 19,8%), while 60 research combined both laboratory and field experiments (12,6%). Honeybees were used in 54 computer-based research (11,3% of articles) (Fig.1B).

Journals: In total analysed articles were published in 202 international journals with impact factor (IF). 111 journals have impact factor (for 2019) higher than 2 and 31 higher than 5. Most frequently, studies using the honeybee as a model appeared in the following journals: Apidologie (40 articles), Journal of Apicultural Research (30), Insects (28), Scientific Reports (23), PLoS ONE (12), Journal of Experimental Biology (12), Environmental Pollution (11) and Ecotoxicology and Environmental Safety (11) (Supp.2).

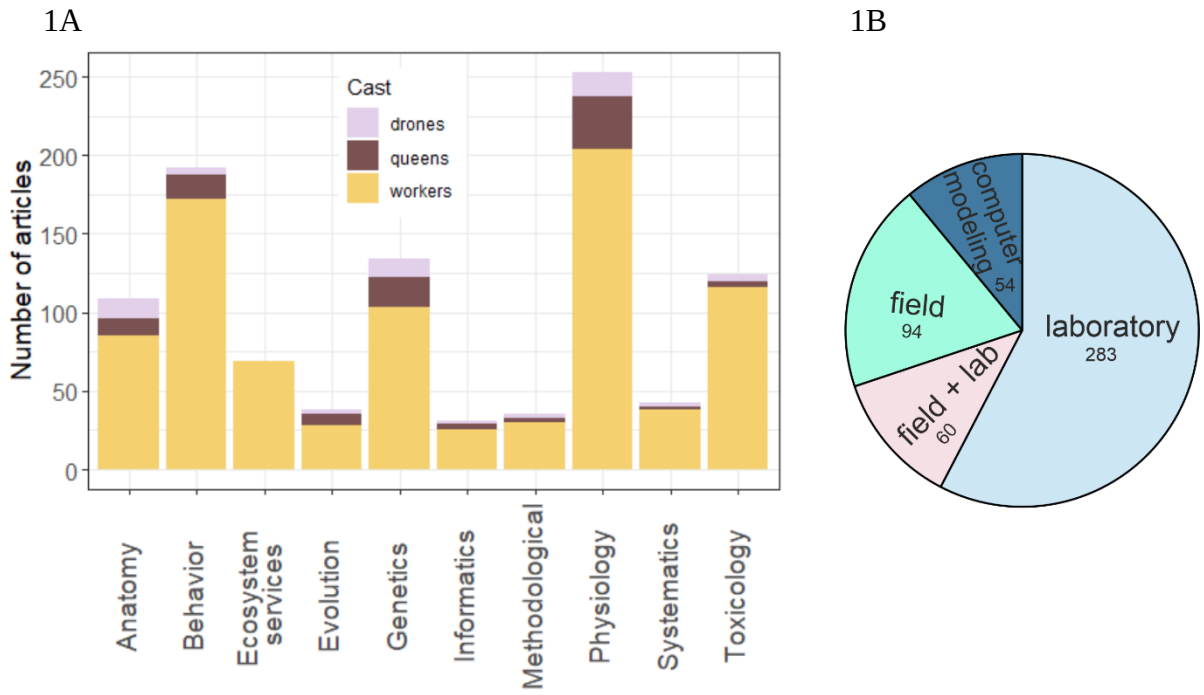


Fig. 1. Results of systematics screen. A – Number of papers with usage of honeybees published in 2020 within defined categories, including division into honeybees' casts (workers, queens, drones). B - Experimental environments used in analysed research (numbers indicate number of articles)

CCA results: The CCA model explained 80,3% of the variability for the first and second component. We established two areas focusing different types of science field journals. Systematics, genetics, anatomy and evolution gather articles form a groups of journals on the upper left side of the Figure 2 (henceforth referred as SGAE). The second big aggregation of journals corresponds to physiology, toxicology and behaviour (henceforth referred as PTB). Three remaining science fields are separated on the plot. Articles assigned to ecosystem services category often referred to bees' pollination behaviour and as such are the closest to behavioural journals. Journals with informatics articles are mostly distanced from biology-related articles. Methodological journals are between biology and technical ones. Eight journals with more than ten articles (marked in orange) are placed in the middle of the plot in PTB group.

Research gathered in group SGAE and toxicology are usually conducted in laboratory environment. Studies from physiology and behavioural journals usually combine laboratory and field experimental environments. Ecosystem services journals more frequently conduct experiments in the field. Computer-based articles are located on the right side of the plot, concerning computer modelling and simulations.

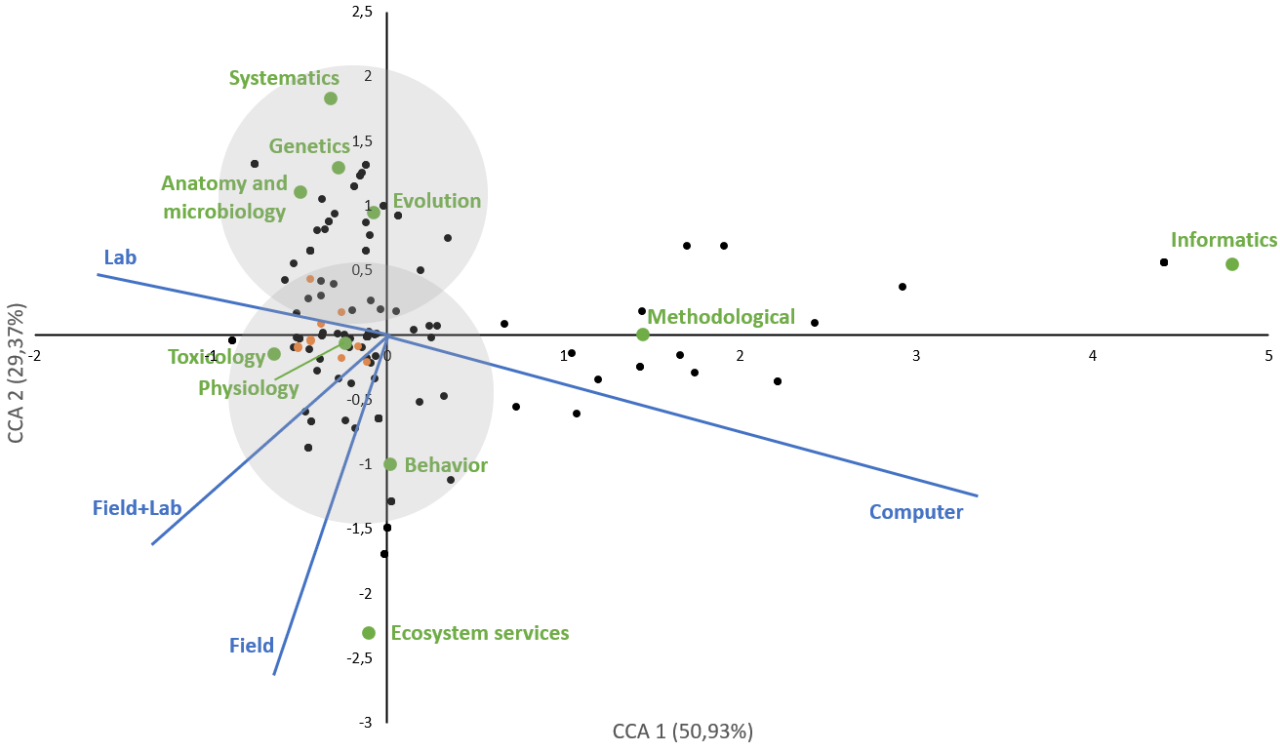


Fig. 2. CCA outcome of journals classification to field sciences. There are four environmental factors (blue lines): experimental environment of study (Lab, Field, Field+lab and Computer modelling or simulations). Distance between black (journal) and green dots (science field) point to how these variables correspond with each other. Orange dots point eight journals that score more than 10 articles included to the systematic review. Grey areas mark groups established by authors.

## Discussion

Here we systematically analysed 474 articles published in 2020 which were using honeybee as a model organism. The articles were analysed for: (i) scientific field covered by the publication, (ii) honeybees' caste, (iii) experimental environment and (iv) journal in which article was published.

Biological fields were divided into nine categories (Anatomy and microbiology, Behaviour, Ecosystem services, Evolution, Genetics, Methodological, Physiology, Systematics and Toxicology), additionally we set non-biological research into category "Informatics", which include also robotics, computer science, and mathematics. Still, there are endless possibilities in defining and classifying scientific fields. Journals and databases, e.g., Web of Science or Scopus, use their own classification of scientific fields. Altogether, it is impossible to develop a coherent classification system which would suits all possible science disciplines [14]. The limited number of classes defined within this project might led to some underestimations of impact of some publications into other disciplines. Nevertheless, we believe that presented here results provide interesting insights into current knowledge and possibilities of usage of honeybees in various scientific fields.

Honeybee is most frequently used in research on physiology, behaviour, genetics and toxicology (Fig. 1A), while fewer research are conducted in systematics and evolution. Majority of analysed articles were classified to more than one scientific field - e.g., studies on honeybees' systematics are largely based on genetics and anatomy, while those relating to ecotoxicology are based on behavioural and physiological tests. Hence, it is more rationale to analyse how scientific fields group together. Our CCA extracted 2 groups of fields: the SGAE group gather systematics, genetics, anatomy and microbiology

and evolution fields, while group PTB gather physiology, toxicology and behaviour. Such outcome is expected, as systematic and evolutionary research are based either on genetic data or anatomical features of organisms. On the other hand, the decrease in the pollinators' abundance, correlated with progressive environmental contamination, forces the emphasis on research in the field of ecotoxicology [15]. Ecosystem services category doesn't group with other biological fields and behaviour is the closest one. Articles classified within ecosystem services often focuses on pollination efficiency, or interspecies interactions which were also assigned as behavioural studies. Informatics, as the most distinct from biological fields is separated also by CCA. These computer-based studies usually use already existing datasets to generate new outcomes. Methodological publications are located intermediately between biological and non-biological fields. These articles also cover important discoveries of new methods and experimental designs as well as improvements of existing ones.

Majority of research with honeybees are conducted on workers. Honeybees' workers are the most numerous caste in a colony and they performed very diverse tasks inside and outside the nest [16]. They are particularly interesting due to advanced social structure (eusociality), division of labour, pollination process, breeding system and constant contact with pollutants, pesticides and heavy metals while forging [16]. Drones, on the other hand, are rarely used. Drones are raised only in strong and healthy colonies and their main task is to pass genes [16]. That is why they are studied mainly in genetics, physiology and anatomy. Also, queens, the second caste involved in reproduction, scored the highest number of articles in the same scientific fields. They were equally well represented in behavioural and evolutionary studies. Such research contributes to knowledge about honeybee breeding system.

Experiments conducted in laboratories are most frequent. This indicates that the honeybee is a well-established model species with standard test procedures developed [10-12] that most studies have been successfully transferred from less controlled field conditions to more stringent laboratory conditions. Most of the research combining field treatment with laboratory work takes place in ecotoxicology studies, where investigating the effects of natural exposure to toxins is particularly important [17]. Many studies were also conducted on already existing data via bioinformatics analysis or modelling. Considering a huge amount of research conducted on honeybees, as well as large datasets, it is highly probable that in near future more research on bees will be developed without the physical use of these organisms [18]. This is of particular importance given the ethical aspects of the use of animals in research.

Research using honeybees are published in broad range of journals, within various fields, differing in scopes and ranks, what confirms that honeybee is valued and well-known model organism. The most popular journals are still specialist journals, focusing on bee-research (Apidologie, Journal of Apicultural Research) or insects in general (Insects, Insects Sociaux), however, many research were published in wide-scope journals which publish innovative, interdisciplinary research (PLoS ONE, Scientific Report, PNAS, Current Biology). These journals are well-known and publish high-quality and innovative research. It is also worth noting that multiple studies were published in specialized journals focusing on ecotoxicology (Environmental Pollution, Ecotoxicology and Environmental Safety), what in turn indicates that the honeybee has become the standard research model in this field. The emerging research with bees in non-biological fields (publications in Bioinspiration and Biomimetics, Biosystems Engineering, Bioelectromagnetics, Vision Research) show wide possibilities of using bees in research which goes far beyond natural sciences. There is an incredible potential in honeybees and surely much can be done and discovered on their basis in the future.

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Supplemental material 1

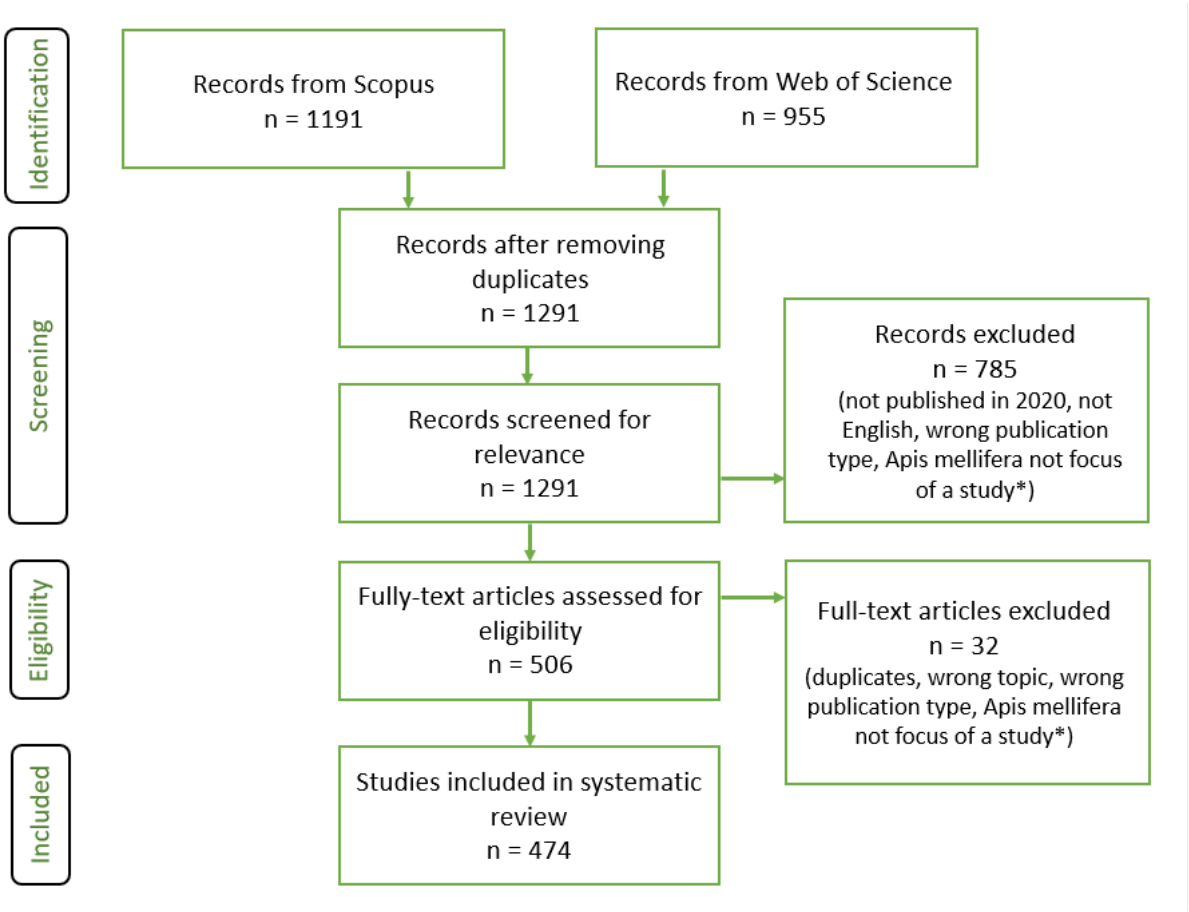


Fig. S1. Diagram PRISMA showing articles flow from searching until including to systematic review scope. * specific exclusion criteria are described in Tab.2

Supplemental material 2

Tab.S1. List of journals included in the systematic review and articles number

| No. | Journal | Articles number | Impact factor for 2019 |
|---|---|---|---|
| 1 | Academic Journal of Manufacturing Engineering | 1 | 0,32 |
| 2 | Acta Agriculturae Scandinavica A: Animal Sciences | 1 | 0,32 |
| 3 | Acta Biomaterialia | 1 | 8,95 |
| 4 | Acta Brasiliensis | 1 | 0,00 |
| 5 | Acta Oecologica | 2 | 1,22 |
| 6 | Acta Scientiarum | 1 | 0,62 |
| 7 | African Entomology | 2 | 0,65 |
| 8 | Agricultural and Forest Entomology | 1 | 1,89 |
| 9 | Agriculture Ecosystems & Environment | 2 | 4,24 |
| 10 | Animal Behaviour | 5 | 2,70 |
| 11 | Annals of parasitology | 1 | 0,77 |
| 12 | Annals Of The Entomological Society Of America | 1 | 1,77 |
| 13 | Apidologie | 40 | 1,83 |
| 14 | Applied and Environmental Microbiology | 1 | 4,79 |
| 15 | Applied Ecology and Environmental Research | 1 | 0,71 |
| 16 | Applied Entomology and Zoology | 2 | 1,11 |
| 17 | Applied Sciences | 2 | 2,84 |
| 18 | Arab Journal of Nuclear Science and Applications | 1 | 0,00 |
| 19 | Archives of Environmental Contamination and Toxicology | 1 | 1,86 |
| 20 | Archives of Insect Biochemistry and Physiology | 1 | 1,54 |
| 21 | Australasian Plant Pathology | 1 | 1,42 |
| 22 | Australian Journal of Botany | 1 | 1,39 |
| 23 | Australian Journal of Crop Science | 1 | 0,72 |
| 24 | Basic and Applied Ecology | 1 | 3,16 |
| 25 | Behavioral Ecology and Sociobiology | 4 | 2,28 |
| 26 | Biodiversity Data Journal | 1 | 1,33 |
| 27 | Bioelectromagnetics | 1 | 2,28 |
| 28 | Bioinspiration and Biomimetics | 1 | 3,13 |
| 29 | Biology | 1 | 3,70 |
| 30 | Biology Letters | 3 | 2,42 |
| 31 | Biology Open | 1 | 3,80 |
| 32 | BioMed Research International | 1 | 2,58 |
| 33 | Biophysics | 1 | 0,00 |
| 34 | BioSystems | 1 | 3,22 |
| 35 | Biosystems Engineering | 1 | 1,81 |
| 36 | BMC Evolutionary Biology | 1 | 3,06 |
| 37 | BMC Genomics | 1 | 3,97 |
| 38 | Brain and Behavior | 1 | 2,53 |
| 39 | Bulgarian Journal of Agricultural Science | 1 | 0,69 |
| 40 | Bulletin of Insectology | 1 | 1,71 |

| | | | |
|---|---|---|---|
| 41 | Bulletin of The National Academy of Sciences of The Republic of Kazakhstan | 2 | 0,21 |
| 42 | Cell and Tissue Biology | 1 | 0,59 |
| 43 | Cell biochemistry | 1 | 4,28 |
| 44 | Cell Reports | 1 | 9,42 |
| 45 | Cellular and Molecular Life Sciences | 1 | 9,26 |
| 46 | Chemistry, Didactics, Ecology, Metrology | 1 | 0,00 |
| 47 | Chemosphere | 5 | 5,78 |
| 48 | Chilean Journal of Agricultural & Animal Sciences | 1 | 0,47 |
| 49 | Chimia | 1 | 0,83 |
| 50 | Communications Biology | 2 | 6,27 |
| 51 | Comparative Biochemistry and Physiology | 2 | 2,23 |
| 52 | Comptes Rendus de L'Academie Bulgare des Sciences | 1 | 0,34 |
| 53 | Computers and Electronics in Agriculture | 3 | 3,86 |
| 54 | Conservation Physiology | 1 | 3,08 |
| 55 | Cryobiology | 1 | 2,05 |
| 56 | Current Biology | 4 | 9,60 |
| 57 | Developmental and Comparative Immunology | 1 | 3,19 |
| 58 | Diversity | 2 | 1,55 |
| 59 | Diversity-Basel | 1 | 2,05 |
| 60 | Ecological Applications | 1 | 4,25 |
| 61 | Ecological Entomology | 1 | 1,85 |
| 62 | Ecological Modelling | 2 | 2,50 |
| 63 | Ecology and Evolution | 3 | 2,39 |
| 64 | Ecosphere | 1 | 2,88 |
| 65 | Ecotoxicology and Environmental Safety | 11 | 4,87 |
| 66 | eLife | 1 | 7,08 |
| 67 | Entomologia Generalis | 1 | 5,63 |
| 68 | Entomology and Applied Science Letters | 1 | 1,91 |
| 69 | Environment International | 1 | 7,58 |
| 70 | Environmental Entomology | 3 | 2,38 |
| 71 | Environmental Pollution | 11 | 6,80 |
| 72 | Environmental Research | 1 | 5,03 |
| 73 | Environmental Science and Pollution Research | 3 | 3,06 |
| 74 | Environmental Sciences Europe | 1 | 5,39 |
| 75 | Environmental Toxicology and Chemistry | 1 | 3,18 |
| 76 | Epidenomes | 1 | 4,11 |
| 77 | Ethology Ecology and Evolution | 1 | 1,58 |
| 78 | European Journal of Neuroscience | 1 | 3,39 |
| 79 | Fresenius Environmental Bulletin | 1 | 0,55 |
| 80 | Frontiers in Behavioral Neuroscience | 2 | 3,10 |
| 81 | Frontiers in Cell and Developmental Biology | 1 | 5,19 |
| 82 | Frontiers in Ecology and Evolution | 2 | 3,26 |
| 83 | Frontiers in Genetics | 3 | 3,79 |
| 84 | Frontiers in Microbiology | 2 | 4,08 |
| 85 | Frontiers in Physiology | 1 | 4,13 |

| | | | |
|---|---|---|---|
| 86 | Frontiers in Plant Science | 1 | 4,30 |
| 87 | Genes and Genomics | 1 | 1,19 |
| 88 | Genes, Brain and Behavior | 1 | 3,45 |
| 89 | Genetics and Molecular Biology | 1 | 1,88 |
| 90 | Genome Biology and Evolution | 4 | 3,46 |
| 91 | Heliyon | 1 | 1,86 |
| 92 | Hormones and Behavior | 1 | 4,45 |
| 93 | IEEE Access | 1 | 3,37 |
| 94 | IEEE Sensors Journal | 1 | 3,08 |
| 95 | IEEE Systems Journal | 1 | 5,28 |
| 96 | Indian Journal of Experimental Biology | 1 | 0,78 |
| 97 | Insect Biochemistry and Molecular Biology | 4 | 1,58 |
| 98 | Insect Science | 1 | 2,79 |
| 99 | Insectes Sociaux | 6 | 1,42 |
| 100 | Insects | 28 | 2,14 |
| 101 | Integrated Environmental Assessment and Management | 1 | 3,44 |
| 102 | International Journal of Advanced Robotic Systems | 1 | 1,48 |
| 103 | International Journal of Agricultural and Statistical Sciences | 1 | 0,28 |
| 104 | International Journal of Cloud Computing | 1 | 0,43 |
| 105 | International Journal of Communication Systems | 1 | 1,32 |
| 106 | International Journal of Comparative Psychology | 1 | 0,85 |
| 107 | International Journal of Environmental Research | 1 | 0,40 |
| 108 | International Journal of Intelligent Engineering and Systems | 1 | 0,20 |
| 109 | International Journal of Wireless and Mobile Computing | 1 | 0,50 |
| 110 | International Transaction Journal Of Engineering Management & Applied Sciences & Technologies | 1 | 0,00 |
| 111 | Iranian Journal of Applied Animal Science | 2 | 0,61 |
| 112 | iScience | 1 | 4,57 |
| 113 | JActa Fytotechnica et Zootechnica | 1 | 0,00 |
| 114 | Journal fur Kulturpflanzen | 2 | 1,22 |
| 115 | Journal of Agricultural Science and Technology | 1 | 0,25 |
| 116 | Journal Of Agricultural Sciences-Tarim Bilimleri Dergisi | 1 | 0,90 |
| 117 | Journal of Ambient Intelligence and Humanized Computing | 1 | 0,40 |
| 118 | Journal of Animal Ecology | 1 | 4,81 |
| 119 | Journal of Apicultural Research | 30 | 4,55 |
| 120 | Journal of Apicultural Science | 6 | 2,38 |
| 121 | Journal of Applied Ecology | 1 | 0,78 |
| 122 | Journal of Asia-Pacific Entomology | 2 | 6,53 |
| 123 | Journal of Biological Rhythms | 1 | 1,17 |
| 124 | Journal of Biosciences | 1 | 3,24 |
| 125 | Journal of Chemical Ecology | 1 | 1,65 |
| 126 | Journal of Comparative Physiology | 1 | 2,12 |
| 127 | Journal of Comparative Psychology | 1 | 1,97 |
| 128 | Journal of Economic Entomology | 4 | 1,94 |
| 129 | Journal of Entomological Research | 2 | 0,21 |
| 130 | Journal of Enzyme Inhibition and Medicinal Chemistry | 1 | 4,31 |

| | | | |
|---|---|---|---|
| 131 | Journal of Evolutionary Biology | 1 | 2,72 |
| 132 | Journal of Experimental Biology | 12 | 3,31 |
| 133 | Journal of Green Engineering | 1 | 1,05 |
| 134 | Journal of Hazardous Materials | 1 | 9,04 |
| 135 | Journal of Insect Behavior | 2 | 2,25 |
| 136 | Journal of Insect Physiology | 9 | 2,51 |
| 137 | Journal of Insect Science | 1 | 1,22 |
| 138 | Journal of Insect Science | 1 | 0,99 |
| 139 | Journal of Invertebrate Pathology | 1 | 1,94 |
| 140 | Journal of Mathematical Biology | 1 | 4,07 |
| 141 | Journal of Proteome Research | 1 | 0,27 |
| 142 | Journal of the Faculty of Agriculture, Kyushu University | 2 | 0,22 |
| 143 | Journal of the Kansas Entomological Society | 1 | 5,00 |
| 144 | Journal of the Mechanics and Physics of Solids | 1 | 6,46 |
| 145 | Journal of the Saudi Society of Agricultural Sciences | 1 | 0,83 |
| 146 | Journal of Theoretical and Applied Mechanics | 1 | 2,36 |
| 147 | Journal of Thermal Biology | 2 | 0,71 |
| 148 | Journal of Thermal Engineering | 1 | 3,46 |
| 149 | Jove-Journal Of Visualized Experiments | 1 | 1,40 |
| 150 | Letters in Applied Microbiology | 1 | 2,17 |
| 151 | Materials Research Express | 1 | 1,61 |
| 152 | Microbial Ecology | 1 | 3,36 |
| 153 | Microbial Pathogenesis | 1 | 2,91 |
| 154 | Microbiological Research | 1 | 5,42 |
| 155 | Microorganisms | 1 | 4,17 |
| 156 | Microscopy Research and Technique | 1 | 2,12 |
| 157 | Mitochondrial DNA Part B | 7 | 0,55 |
| 158 | Molecular and Cellular Probes | 1 | 1,87 |
| 159 | Molecular and Cellular Proteomics | 1 | 5,91 |
| 160 | Molecular Biology And Evolution | 2 | 16,24 |
| 161 | Molecular Ecology | 1 | 6,19 |
| 162 | mSystems | 1 | 5,85 |
| 163 | Nature Communications | 2 | 14,92 |
| 164 | Nature Sustainability | 1 | 9,65 |
| 165 | Neotropical Entomology | 2 | 1,33 |
| 166 | Neural Computing and Applications | 1 | 4,77 |
| 167 | Neurobiology of Learning and Memory | 1 | 3,24 |
| 168 | Oecologia | 1 | 2,65 |
| 169 | Online Journal of Animal and Feed Research | 1 | 1,53 |
| 170 | OnLine Journal of Biological Sciences | 1 | 0,45 |
| 171 | Pacific Science | 1 | 0,82 |
| 172 | Pakistan Journal of Zoology | 1 | 0,92 |
| 173 | Palynology | 1 | 1,33 |
| 174 | PeerJ | 3 | 2,98 |
| 175 | Peer-to-Peer Networking and Applications | 1 | 2,79 |
| 176 | Pest Management Science | 3 | 3,75 |

| | | | |
|---|---|---|---|
| 177 | Pesticide Biochemistry and Physiology | 3 | 2,75 |
| 178 | Pesticide Research Journal | 1 | 1,10 |
| 179 | PLoS Genetics | 1 | 5,91 |
| 180 | PLoS ONE | 12 | 3,24 |
| 181 | Polish Journal of Veterinary Sciences | 1 | 0,52 |
| 182 | Proceedings of the National Academy of Sciences of the United States of America | 7 | 11,21 |
| 183 | Proceedings of the Royal Society B | 5 | 5,35 |
| 184 | Revista Chilena de Historia Natural | 1 | 1,41 |
| 185 | Revista Ciencia Agronomica | 1 | 0,50 |
| 186 | Revista Mexicana de Ciencias Pecuarias | 1 | 1,33 |
| 187 | Royal Society Open Science | 1 | 2,96 |
| 188 | Science | 1 | 14,14 |
| 189 | Science Advances | 1 | 7,96 |
| 190 | Science of the Total Environment | 9 | 41,85 |
| 191 | Scientific Reports | 23 | 4,00 |
| 192 | Semina: Ciencias Agrarias | 1 | 0,50 |
| 193 | SIAM Journal on Applied Mathematic | 1 | 1,55 |
| 194 | Sociobiology | 5 | 0,95 |
| 195 | Soft Matter | 1 | 3,40 |
| 196 | Theoretical Population Biology | 1 | 1,26 |
| 197 | Turkiye Entomoloji Dergisi | 2 | 0,57 |
| 198 | Urban Ecosystems | 1 | 2,55 |
| 199 | Veterinaria Y Zootecnia | 1 | 0,47 |
| 200 | Veterinary Sciences | 3 | 1,47 |
| 201 | Viruses | 1 | 5,05 |
| 202 | Vision Research | 1 | 2,61 |

## Reviewer: Piotr Łukasik

In this literature review, the authors summarize their findings on the contents, classification, and published journals of 2020 articles focused on some aspect of the honeybee (Apis mellifera) biology. Honeybee is clearly a significant organism from the ecological, economical and societal perspective, important also as a model organism, and I can definitely see the value in a systematic review of the main research directions. The authors have made a substantial effort to select and screen large numbers of published studies in a short timeframe, and I applaud the effort.

My overall feeling, despite the topics' significance and the authors' efforts, was that the analyses and their interpretation were somewhat superficial, and have not led to clear and significant conclusions, thus leaving little of the "take home" message.

First, I would like to see a bit more thorough introduction to the significance of honey bees to the modern society and scientific community. What is their importance as model organisms? What the most important research directions have been? Having some specific examples --- e.g., that the bees' pollination services are estimated at X billions dollars per year, that they are a reference organisms in standardized toxicity testing, or that honeybee gut bacteria have become a model of host-microbe interactions --- would have made the introduction much more effective, in my opinion.
Also, what were the authors expectations regarding the primary findings? Have there been any prior reviews of the primary research directions on bees?

My other concern is about the choice of articles for inclusion. Some of the criteria for the article inclusion were straightforward (primary research article, 2020, English) - but then, they then chose to exclude some of the research fields/directions, and I have felt that the choice was quite arbitrary. Why articles focused on biotic interactions (predators, parasites, pathogens, veterinary aspects...) were excluded? Were there any articles about societal significance - like, the estimated value of honeybee services, the use of honeybees in arts, public perception of bees, etc. - and if so, were they included, and how were they classified? These uncertainties have cast some doubt on the reliability of the article choice, and thus, the conclusions.

I would like to see more information on how the articles were classified across fields. How often were articles classified to several different fields? I would like to see an analysis of these data. Perhaps through Gantt charts? Also, how publication impact varies across fields? Providing some specific examples, for example, on what the highest-IF article was about, would have also been helpful, in my opinion. I would like to see a more thorough description of the CCA, in a way accessible to a reader who is not an expert in multidimensional analyses. What data exactly was used as input? What were the outputs - these components? What do they mean?

In the Discussion, the authors repeat some of the methods and results, and write about certain aspects of their findings. I would like to get a broader perspective, including comprehensive comparison with other similar studies/systems, the discussion of trends, emerging research directions, authors' predictions on how the dominant directions change, etc. Such a discussion, with a clear "take home" message, would give the article much more of a lasting impact.

The article would benefit from a comprehensive English language revision. I would recommend the use of a grammar editing software such as Grammarly; in my experience, it makes a huge difference to the quality of academic writing by non-native English speakers.

Specific comments:
The title is innovative, but does not explain what the article is about. Possible wording could include "survey of the primary research domains"?

Key words are lacking!

Formatting was lacking. Applying clearly different "Heading" formatting to Introduction, Methods, etc. would give the article a much better outlook. Bolding certain portions of methods and results would also make it easier to follow.
Intro: you should clarify early on what you are referring to as a bee. Apis mellifera only?

Line 35 "Consequently, honeybee had been domesticated..." - I feel this is a logical error: domestication is not an obvious consequence of the common use of bee products.

Line 42: "in variety of biological and non-biological fields" - please provide examples!

Line 43: "However, how widely the honeybee is used, has not been analyzed yet. " - it is not at all clear that the authors mean the primary research directions on this insect group. Use more precise wording, perhaps along the lines of, "However, the full range and relative importance of research fields that use honeybee as the model has not been comprehensively investigated."

Line 44: "The extensive use of bees..." - same concern as above.

Line 44: "standard research methods" - for what types of research?

Line 45: "presumably research on bees can be performed without using bees" - this is an extremely bold and controversial statement. I'm sure it would be possible to do in some fields, but in others (molecular biology, genomics, microbiomics...) it would be very hard or impossible to do high-profile work without the generation of new data.

Line 51: "often chose only ‘catchy’ research" - use more precise wording.

Lines 80-82: "when the research is conducted on honeybees, one of the names used in search string is always used in the title or keywords." - I would disagree: have quickly found studies focused on bee microbiomes where this is not the case.

Line 85: "We conducted abstract screening in Rayyan". I don't know Rayyan, and have no clue what it does. As it seems quite important for your project, please explain.

Table 1: The second column, with the value "E" everywhere, is not useful. Remove and rename the table as "Criteria for exclusion..."? Also, why use the table and not just explain in the main text that you have only included primary research articles written in English and published in 2020, excluding ...?

Line 96: "(iii) honeybees’ cast" - I struggled to figure out what the authors mean

Line 119: You should start from providing some basic statistics on your data. Like, how many articles were considered in total?

Line 142: "The CCA model explained 80,3% of the variability for the first and second component." - What are these components?

Lines 189-190: It is disputable as to whether the extent of environmental pollution, as a whole, goes up. Also, it would be helpful to stress that honeybees are used as standard reference organisms in toxicity testing.

Table S1. Clarify what type of Impact Factor you are providing. Also, according to the table, the journal "Science" has impact factor of 14, whereas "Science of the Total Environment" - 42... That does not seem right. Most other values I inspected sound about right... but how reliable the table is as the whole?

## Reviewer: Agata Burzawa

Introduction: At the first look I was attached by fancy tricky title. The general idea of the systematic review was new and novelty. Questions were well considered, and probably they fully answered for general idea. From evolutionary point of view, the questions were good established. Nowadays when bees are generally in decline it is extremally important to recognize what drives them to this dramatic state and which of the factors is the most dangerous and harmful for the bees. I understood that the researchers from this review want to analyse where the knowledge gap is and fulfil it for the future. This is the only one information which was missing for me- the impact of this review on the future studies. Authors very well described the aim of conducting this kind of systematic review. The only one part which was missing is the summary. But I can imagine that the authors had enough work to do with other parts of this systematic review.

Methods: In this part the number of gathered data on each level should be included. In the end, the reader has no idea how huge part of knowledge and studies are dedicated to the pollinators especially bees. I suggest include this information only in brackets. Not to emphasize it a lot. I think putting this information in supplementary materials is not a good idea. Sometimes it is not visible for the readers, but it is also important information.
The questions were very well established in the part dedicated for full-text screening. It fully answered asked researched question.
Part where the authors were analysing CCA in the methods was really difficult to understand. Maybe more explanations are needed as well this really complicated figure 2. I can imagine that it is extremally hard to show such a complex research idea, but sometimes it is confusing. Especially when you are clamming in the discussion that: “Still, there are endless possibilities in defining and classifying scientific fields”. However this part of discussion with was dedicated for this analysis was exhausting enough. But still I can believe it was probably the most demanding part of this work.
I would suggest to exclude one table and reverse it with a text.
Discussion: The only one information that is missing for me is weather the outcomes followed your idea from the beginning or no. The results were really interesting, we learnt from that a lot, but on the other hand were also surprising. I assumed that argumentation of this outcomes will be more extended.
What’s more, all other aspects that were searched during full-test screening was fully analysed and discussed.
Moreover, I think this idea of attaching other supplementary materials such as “List of journals included in the systematic review and articles number” with impact factor, was really good.
Overall the general idea of this study and also way of explaining the hypothesis was great. I think we can learn from this type of systematic review a lot. The whole structure of this systematic review was also clear. For such a short period you did an excellent work. Really great job!

## Reviewer: Junchen Deng

Ostap-Chęć et al. systematically reviewed scientific publications in 2020 using honeybees as model species. Through abstract and full-text screening, they included 474 publications in this study. From the screened articles, they extracted information about 1) field of study (ten categories), 2) research types (field, lab, field+lab, and modeling), 3) the studied honeybee castes (worker, drone, and queen), and 4) the scientific journal where the study was published. The authors found that the majority of studies were concerned about the physiology and behavior of honeybees. The statistical analysis identified two groups of study fields, within which the studies are more closely associated with each other. They also found that the honeybee workers were the most studied caste and most of the studies were conducted in the lab. The list of scientific journals includes some areas that are outside of natural science, indicating a great and broad potential of honeybees.

General comments:
This study gives a comprehensive review of honeybees as model species in scientific research in 2020 and shows that honeybees were popular and important in fields both inside and outside natural science. The methods were well-presented, and the results were properly interpreted and discussed. However, the text is a bit too much (~2534 words excluding titles, tables, references, and supplementary materials). I also faced some difficulties in understanding the statistical analysis ( Canonical Correspondence Analysis, CCA) and its results, which needs to be elaborated more in the text. In addition, the grammar of the text is often inaccurate, which makes the article hard to read through. These issues will be pointed out in detail in the next section and should be corrected in the revised version.

Comments on Introduction:
well-written and -structured introduction!

Comments on Materials and Methods:
*__Line 65__: in the sample section, you wrote "honeybee *Apis mellifera*". To my understanding, "honeybee" is a common name for species from the genus *Apis*, and you included not only *A. mellifera* but also other honeybees in this study. So, the honeybee here should not be specified to *A. mellifera*, right?

*__Line 113__: the section about Canonical Correspondence Analysis (CCA). I have difficulties in understanding the methods here as a non-expert in the field of statistics. Please give more detailed information about this analysis. For example, what does CCA generally do, what parameters does CCA take into, how will CCA use group factor and environment variable (I presumed from the text) to calculate the relationship between variables. In addition, at __Line 142__, you mentioned "The CCA model explained 80.3% of the variability for the first and second component". What are the first and second components? How were they calculated? What can "80.3% explained variability" tell us?

Comments on Results:
*__Line 118__: at the beginning of the result section. I suggest adding one sentence about how many articles you found before and after the screening. I know that you have the information in Figure S1, but it is good to mention it in the results.

*__Line 142__: CCA results. The issue was mentioned in the previous section.

*__Line 149, 150__: when interpreting Figure 2, you used the words “distanced”, “between” and “in the middle”. I would like to know what the distance between dots means in Figure 2. In the caption of Figure 2, you mentioned that “ Distance between black (journal) and green dots (science field) points to how these variables correspond with each other”. But what does a great or a short distance mean? In addition, the black dots in Figure 2 represent scientific journals, but readers cannot see which dot is which journal. This makes sentences like “Journals with informatics articles are mostly distanced from biology-related articles” very unclear and confusing. Please find a way to interpret the figure clearer.

Comments on Discussion:
*__Line 171__: “nine categories” should be changed to “ten categories” according to Table 2.

*__Line 202__: Drones. I am not familiar with bee castes, so I was confused when seeing “drones” for the first time. I think it would be better if you could use one sentence to introduce this term. For example, drones are male honeybees dedicating only to mate with the queen.

*__Line 222__: I think the statement “These journals are well-known and publish high-quality and innovative research” is too general. Not every publication from the journals you mentioned is good in quality, not to mention that the list includes PLoS ONE and Scientific Report, from which the quality of the publications is inconsistent.

Comments on Figures and Tables:
*__Figure 1__: great figure! Only the “cast” should be “caste” in the legend.
*__Figure 2__: needs more interpretation as mentioned in the previous section.
*__Table S1__: could you add the commonly used abbreviation to some journals? Such as “PNAS”

General comments on the text:
As I mentioned at the beginning, there are many grammatical errors in this paper. The grammatical tense of many sentences is also inaccurate. I highly recommend using Grammarly as an extension to your browser to check the grammar of your text. When I imported this document into google docs, Grammarly gave me more than 190 suggestions on potential grammatical errors, which is too many for a paper. Please read through the paper and check the grammar again. Here I will list a few errors.
*__Line 42__: “wider recognition is science”. “Is” should be “in”
*__Line 98__: “more the one research field”. “The” should be “than”
*__Line 124__: “cast” should be “caste”
*__Line 178__: “into” should be “on”
*__Line 192__: “interspecies” should be “interspecific”
*__Line 202__: “forging” should be “foraging”

Report - final version

# Bee a science star – a systematic review of the primary research domains on *Apis mellifera*

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**Summary**

Honeybee (*Apis mellifera*) is a widely used model organism. Traditionally, research on honeybees had been focused on pollination, the evolution of eusociality, and beekeeping. Nowadays, the honeybee has become a model in a variety of biological and non-biological fields of science. Here, we performed the systematic screening of articles published in 2020, in which the honeybee was the main research object. The analysis aimed to check: which science fields currently use honeybee as a model organism, what type of research (field, laboratory, computer modelling) is used most frequently and on which bees' caste. Moreover, we investigated studies on honeybees in terms of journals in which they were published. Results demonstrate, that extracted publications most frequently concern physiological and behavioural fields, however, the majority of studies were classified into more than one category. Therefore, we analysed how articles could be grouped and concluded that research most often combines systematics, genetics, anatomy and evolution, or toxicology, physiology, and behaviour. As expected, honeybees' workers are used most frequently, and most of the researches are conducted in laboratories. In total, analysed articles were published in 201 journals with a broad range of specialisations.

**Keywords:** honeybee, Apis mellifera, systematic review, science, model organism

## 1. Introduction

The honeybee has been a well-known species for years, both as a breeding species that supply bee products and as a model in research. Initially, scientists' attention had been directed towards their advanced social structuring, which led to the development of research on the evolution of eusociality [1,2], behavior and communication in animals [3-5], or plant pollination [6]. However, more recently, the honeybee is gaining much wider recognition in science, becoming a model species in such fields as ecotoxicology [7], host-microbiome interaction [8], or alcoholism [9]. Moreover, research on honeybees is not limited to biological fields, as they are also used in informatics [10], robotics [11], or computing technology [12]. However, although the honeybee has become a common model organism, the full range and relative importance of research fields that use honeybee have not been comprehensively investigated.

The growing number of studies on honeybees resulted in the development of many standard research methods [13-15], which in turn enabled the transfer of many experiments from the field to laboratories. The rapid progress of science generates so much data, that some tests could be done using only computer modelling or programming. An important aspect of research on honeybees is also - where such results are published. Journals are characterized by a different degree of specialization and range. Publications in the most prestigious international journals with high impact factors require not only the highest research quality and innovation but also the broad scientific importance of discoveries. Moreover, journals differ in the scope of published studies, thus some of them reach a broader audience or more specialized scientists.

Our study aimed to follow scientific publications from 2020 in which the honeybee (only *A.mellifera* species) was the research model and analyze these studies in terms of the field of science, type of research, bee caste, and the journal in which it was published. This systematic review allowed us to answer the following questions: [Q1] which science fields currently use honeybee as a model organism, [Q2] which study type (experimental, laboratory, computer modelling) is used most often [Q3] which honeybees' castes are most frequently used, and [Q4] which journals publish research using honeybees. Answering these questions will allow us not only to illustrate the current trends in honeybee research but also to see which areas and types of study are poorly researched and require more attention.

## 2. Materials and Methods

Classification of articles for systematic review was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Fig.1A). Initially, we established SPIDER statement to support our research string.

| | |
|---|---|
| **S**ample: | All research articles conducted with the usage of honeybee *A.mellifera* |
| **P**henomena of **I**nterest: | Variety of biological fields which use honeybee as a model species |
| **D**esign: | NA (we include all research papers) |
| **E**valuation: | NA (we do not classify papers based on outcome) |
| **R**esearch type: | Original research articles |

Then, the search strings were run through two science databases:
1) Web of Science:
TITLE(honeybee*OR"honey bee*"OR"Apis mellifera"OR"A. mellifera")OR AUTHOR KEYWORDS(honeybee*OR"honey bee*"OR"Apis mellifera"OR"A. mellifera")AND PUBLICATION YEAR(2020)AND DOCUMENT TYPE(Articles)
2) Scopus:
(TITLE(honeybee*OR"honey bee*"OR"Apis mellifera"OR"A. mellifera")OR KEY(honeybee*OR "honey bee*"OR"Apis mellifera"OR"A. mellifera"))AND(LIMIT-TO( PUBYEAR,2020)) AND(LIMIT-TO(DOCTYPE ,"ar"))
Abstracts were excluded from the search string, since multiple papers use honeybees as a comparison or mention them as an example, even if the research does not concern honeybees. Simultaneously, when the research is conducted on honeybees, one of the names used in the search string is always used in the title or keywords.

### 2.1. Abstract screening

Next, we deduplicated extracted articles and conducted abstract screening in Rayyan [16]. All authors performed independent abstract screening of all articles. As we were interested only in the newest data, only original scientific articles published in 2020 and written in English were considered. We assumed that such a collection of articles is representative of a whole set of research using honeybee. We excluded review articles, book chapters, and grey literature. We also exclude articles concerning: the biology of honeybees' pests, pathogens, and predators; applications and parameters of bees' products (i.a. pollen, propolis, honey); bees' biodiversity; beekeeping; and studies on other species from genus *Apis*.

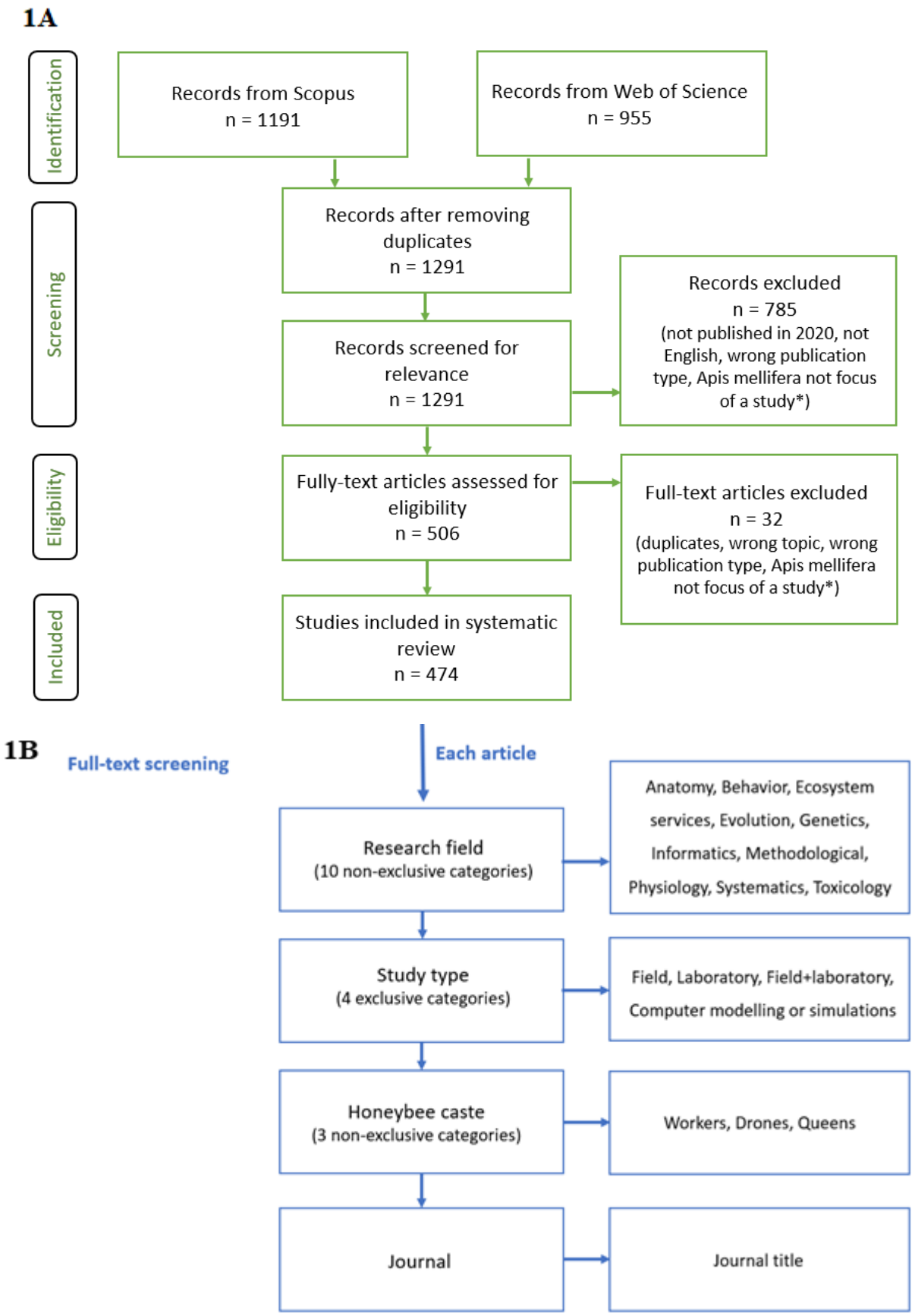


Fig. 1. Search flow **A.** Diagram PRISMA (green part) showing articles flow from searching until including to systematic review scope. * specific exclusion criteria are described in 2.1. paragraph. **B.** Full-text screening procedure.

2.2. Full-text screening

The articles qualified for full-text screening were randomly divided between all authors, where one article was screened by one person. Four aspects of each article were analyzed: research field; type of study; honeybees' caste; and journal in which the research was published. Ten non-exclusive categories of research fields were established (Tab.1). Classification of study type was applied to the experimental or observational part of the research. Articles were classified as laboratory, field, laboratory, and field or computer modelling (Fig.1B). In the case of all treatments being performed in the field, followed by sample analysis conducted in a laboratory, the article was still qualified as a field study. The articles were also grouped according to the honeybees' caste (workers, drones, or queens) used in the experiments.

Tab. 1. Categories of research fields

| Category | Thematic scope |
|---|---|
| Anatomy and microbiology | body parts, organs, gut microbiota, symbionts, morphology |
| Behavior | learning, memory, addictions, communication |
| Ecosystem services | pollination, different environments, conservation biology |
| Evolution | evolution of eusociality, division of labor, inheritance |
| Genetics | DNA and RNA sequencing, mitochondrial DNA, microsatellites, |
| Informatics | non-biological, computer-based research, algorithms, simulations, robotics, mathematic |
| Methodological | comparison of the efficiency of methods, protocols |
| Physiology | endocrinology, immunology, organs functioning, reproduction, growth, and development |
| Systematics | subspecies, taxonomy |
| Toxicology | pesticides, heavy metals, air pollutants, aromatic hydrocarbons, insecticides |

2.3. Canonical Correspondence Analysis

We performed Canonical Correspondence Analysis (CCA) in the Past 3 software to analyse dependencies between collected data: journal, science fields (group factor) and study type (environmental variable). CCA is a multivariate method of data relationships analysis. In our study, CCA shows the similarity of journals in the case of given factors. We analyse the influence of two main components CCA1 and CCA2. Each of them is a different way of describing data variability.

## 3. **Results**

We systematically analysed 474 articles published in 2020 which were using honeybee as a model organism.

**Research fields**: Most frequently the articles were assigned to category physiology (253 articles) and behaviour (192). More than 100 articles were also concerned about genetics (134), toxicology (124) and anatomy (109). Ecosystem services were referred to in 69 articles, the least frequently articles were involving systematics (43) and evolution (38). There were 35 methodological articles and 31 non-biological (including informatics, robotics, computer science, and mathematics) (Fig.2A).

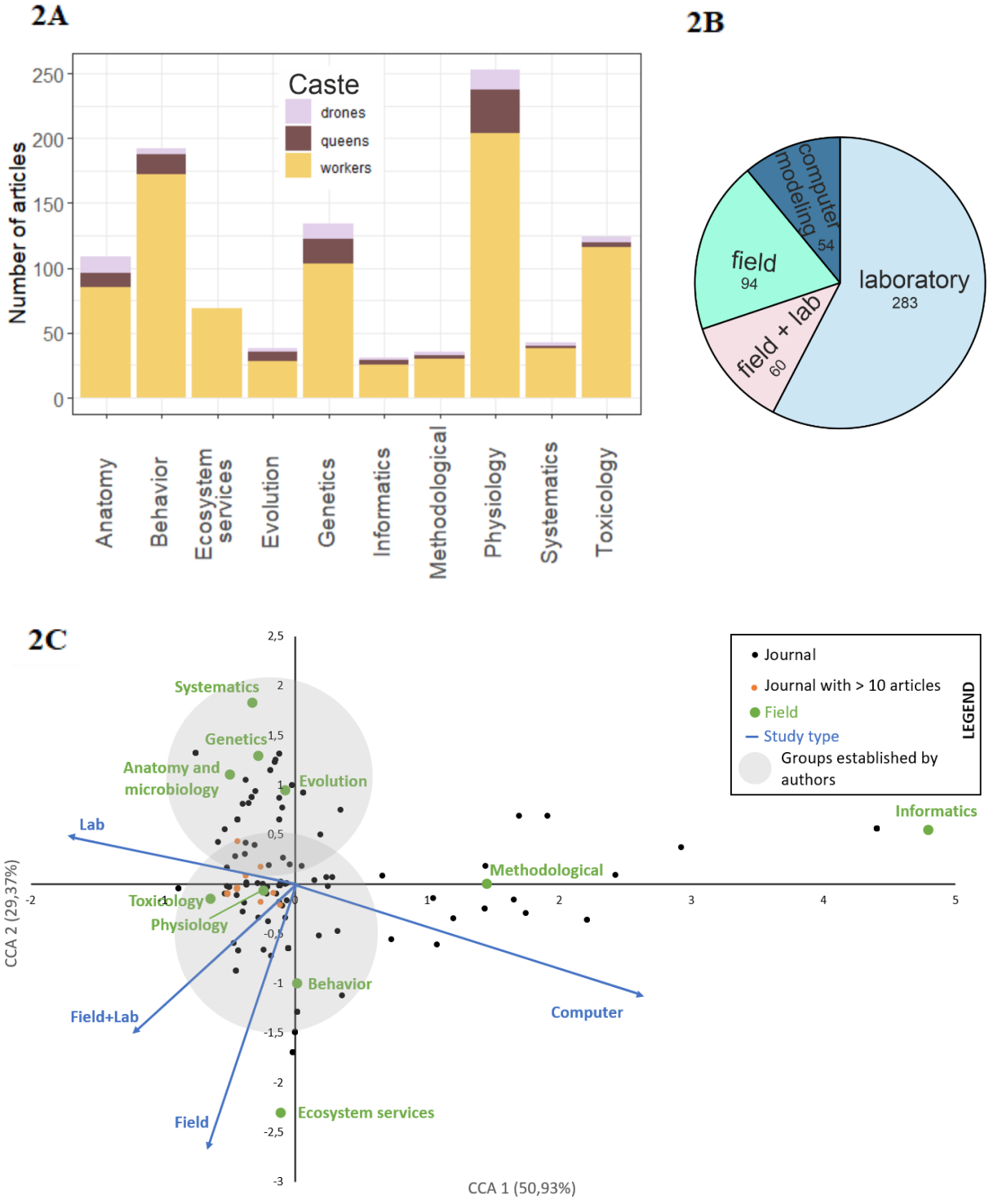


Fig. 2. Results of systematics screen. **A**. Number of papers with the usage of honeybees published in 2020 within defined categories, including division into honeybees' casts (workers, queens, drones). **B.** Experimental environments used in analysed research (numbers indicate the number of articles). **C.** CCA outcome of journals classification to science fields. The short distance between black dots (journals) shows the similarity of those. Distance between black and green dots (science field) points to how these variables correspond with each other. There are four environmental factors – study type (blue lines). Only grey areas mark groups established by authors.

**Study type**: More than half of the analysed research was conducted only in laboratories (283 articles, 59,7%). One-fifth of experiments or observations were conducted in a field (94 articles, 19,8%), while 60 research combined both laboratory and field environments (12,6%). Honeybees were used in 54 computer-based research (11,3% of articles) (Fig.2B).

**Honeybees' caste**: The vast majority of research are conducted on honeybee workers (440 articles, 92%), while queens were used in 50 articles (10,5%) and drones only in 32 (6,7%) (Fig.2A)

**Journals**: In total analysed articles were published in 201 international journals with impact factor (IF). 111 journals have an impact factor (for 2019) higher than 2 and 31 higher than 5. Most frequently, studies using the honeybee as a model appeared in the following journals: Apidologie (40 articles), Journal of Apicultural Research (30), Insects (28), Scientific Reports (23), PLoS ONE (12), Journal of Experimental Biology (12), Environmental Pollution (11) and Ecotoxicology and Environmental Safety (11) (Supp.1).

**CCA results:** The CCA explained 80,3% of the variability for the CCA1 and CCA2 (Fig.2C). We established two areas gathering interconnected research fields: physiology, toxicology and behaviour (henceforth referred to as PTB) and systematics, genetics, anatomy and evolution (henceforth referred to as SGAE). PTB group is the most numerous and includes all journals where more than 10 articles are published. There are some journals belonging to PTB and SGAE. Three remaining science fields are separated on the plot. Articles assigned to the ecosystem services category often referred to bees' pollination behaviour and as such the closest to behavioural journals. Journals with informatics articles are mostly distanced from biology-related articles. Methodological journals are between biology and technical ones. SGAE group and toxicology are usually conducted in laboratories. Studies from physiology and behavioural journals usually combine laboratory and field environments, while ecosystem services more frequently conduct experiments in the field. Computer-based articles are located on the right side of the plot.

**Discussion**

The honeybee is most frequently used in research on physiology, behaviour, genetics and toxicology (Fig. 1A). However, the majority of analysed articles were classified into more than one scientific field, hence, we analyse how scientific fields group together. From CCA we extracted 2 groups: the SGAE group gather systematics, genetics, anatomy and microbiology and evolution fields, while group PTB gather physiology, toxicology and behaviour. Such outcome is expected, as systematic and evolutionary researches are based either on genetic data or anatomical features of organisms. The decrease in the pollinators' abundance, correlated with progressive environmental contamination, forces the emphasis on ecotoxicology [17] which main research methods are physiological and behavioural tests. The ecosystem services category does not group with other biological fields and behaviour is the closest one. Articles classified within ecosystem services often focus on pollination efficiency or interspecies interactions which were also assigned as behavioural studies. Informatics, as the most distinct from biological fields, is separated also by CCA. These computer-based studies usually use already existing datasets to generate new outcomes. Methodological publications, which cover descriptions of new methods and experimental designs as well as improvements of existing ones, are located intermediately between biological and non-biological fields (Fig.2C). It highlights that the methodology of working with bees is constantly improved and adapted to modern standards, and there are multiple tools already developed for various purposes. The systematics contains relatively little research, while anatomy still accounts for a large share. This fact is most likely driven by studies on the microbiome, symbiotes or host-microorganism interactions. The

honeybee has gained great popularity in research into biodiversity, and interactions with microorganisms, as the article concerning this subject was published in the prestigious journal Science.

The majority of research with honeybees are conducted on workers. They are the most numerous caste and they performed very diverse tasks inside and outside the nest [18]. Workers are particularly interesting due to advanced social structure, division of labour, pollination, breeding system and contact with pollutants, pesticides and heavy metals while foraging [18]. Drones (males), however, are rarely used. Their small share in research is not surprising, as they are present in colonies only for a short period of the season, and their only function is to mate with the queen [18]. Hence, most research on both drones and queens concern physiology and genetics. Surprisingly little research on queens is performed in the ecotoxicology field. Indeed, it is mainly workers who forage for food the most exposed, but the toxins and pollutants brought to the hive affect also other members of the colony and the queens seem to be the most important of them, as the strength and size of the colony depend on her welfare, health and reproductive predispositions [18].

Experiments conducted in laboratories are most frequent. This indicates that the honeybee is a well-established model with standard protocols [13-15] that most studies have been successfully transferred from less controlled field conditions. Most of the researches combining field and laboratory work that take place in ecotoxicology where investigating the effects of natural exposure to toxins are particularly important [19]. Many studies were also conducted on already existing data via bioinformatics analysis or modelling. Considering a huge amount of research conducted on honeybees, as well as large datasets, it is highly probable that in near future more research on bees will be developed without the physical use of these organisms [20]. This is of particular importance given the ethical aspects of the use of animals in research.

Research using honeybees are published in a broad range of journals, within various fields, differing in scopes and ranks, which confirms that the honeybee is a valued model organism. The most popular are still specialist journals, focusing on bee-research (Apidologie, Journal of Apicultural Research) or insects in general (Insects, Insects Sociaux), however, many research were published also in wide-scope journals (PLoS ONE, Scientific Report, PNAS, Current Biology). Also multiple studies were published in specialized journals focusing on ecotoxicology, which indicates that the honeybee has become the standard research model in this field. The emerging research with bees in non-biological fields (publications in Bioinspiration and Biomimetics, Biosystems Engineering, Bioelectromagnetics) show wide possibilities of using bees in research that goes far beyond natural sciences.

Our analysis extracted the most intensively studied fields and caste, which entails the existence of the largest datasets. Those areas can in near future be analyzed by multi-factor meta-analyzes to make broad conclusions even without conducting empirical experiments. Systematic categorization of research can also be useful to explore the newest discoveries within the field of interest.

Although every effort has been made to make our analysis objective, it has some limitations. We are aware that chosen domain categories constitute a simplification and a limited number of classes might lead to some underestimations of some publications' impact on other disciplines. However, it is impossible to develop a coherent classification system that would suit all possible science disciplines [21]. We also take into consideration that some publications could be overlooked due to exclusion of abstracts. Nevertheless, we believe that the presented analysis provides interesting insights into current knowledge and possibilities of usage of honeybees in various scientific fields.

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Supplemental material 1

Tab.S1. List of journals included in the systematic review and articles number

| No. | Journal | Articles number | Impact factor for 2019 |
|---|---|---|---|
| 1 | Apidologie | 40 | 1,83 |
| 2 | Journal of Apicultural Research | 30 | 2,38 |
| 3 | Insects | 28 | 2,14 |
| 4 | Scientific Reports | 23 | 4,38 |
| 5 | Journal of Experimental Biology | 12 | 3,31 |
| 6 | PLoS ONE | 12 | 3,24 |
| 7 | Ecotoxicology and Environmental Safety | 11 | 4,87 |
| 8 | Environmental Pollution | 11 | 6,80 |
| 9 | Journal of Insect Behavior | 9 | 2,25 |
| 10 | Science Advances | 9 | 14,14 |
| 11 | Mitochondrial DNA Part B | 7 | 0,55 |
| 12 | PNAS | 7 | 11,21 |
| 13 | Insectes Sociaux | 6 | 1,42 |
| 14 | Journal of Apicultural Science | 6 | 0,78 |
| 15 | Animal Behaviour | 5 | 2,70 |
| 16 | Chemosphere | 5 | 5,78 |
| 17 | Proceedings of the Royal Society B | 5 | 5,35 |
| 18 | Sociobiology | 5 | 0,95 |
| 19 | Behavioral Ecology and Sociobiology | 4 | 2,28 |
| 20 | Current Biology | 4 | 9,60 |
| 21 | Genome Biology and Evolution | 4 | 3,46 |
| 22 | Insect Biochemistry and Molecular Biology | 4 | 1,58 |
| 23 | Journal of Economic Entomology | 4 | 1,94 |
| 24 | Biology | 3 | 3,70 |
| 25 | Computers and Electronics in Agriculture | 3 | 3,86 |
| 26 | Ecology and Evolution | 3 | 2,39 |
| 27 | Environmental Entomology | 3 | 2,38 |
| 28 | Environmental Science and Pollution Research | 3 | 3,06 |
| 29 | Frontiers in Genetics | 3 | 3,79 |
| 30 | PeerJ | 3 | 2,98 |
| 31 | Pest Management Science | 3 | 3,75 |
| 32 | Pesticide Biochemistry and Physiology | 3 | 2,75 |
| 33 | Veterinary Sciences | 3 | 1,47 |
| 34 | Acta Oecologica | 2 | 1,22 |
| 35 | African Entomology | 2 | 0,65 |
| 36 | Agriculture Ecosystems & Environemt | 2 | 4,24 |
| 37 | Applied Entomology and Zoology | 2 | 1,11 |
| 38 | Applied Sciences | 2 | 2,84 |
| 39 | Bulletin of The National Academy of Sciences of The Republic of Kazakhstan | 2 | 0,21 |

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|---|---|---|---|
| 40 | Communications Biology | 2 | 6,27 |
| 41 | Comparative Biochemistry and Physiology | 2 | 2,23 |
| 42 | Diversity | 2 | 1,55 |
| 43 | Ecological Modelling | 2 | 2,50 |
| 44 | Frontiers in Behavioral Neuroscience | 2 | 3,10 |
| 45 | Frontiers in Ecology and Evolution | 2 | 3,26 |
| 46 | Frontiers in Microbiology | 2 | 4,08 |
| 47 | Iranian Journal of Applied Animal Science | 2 | 0,61 |
| 48 | Journal fur Kulturpflanzen | 2 | 0,25 |
| 49 | Journal of Asia-Pacific Entomology | 2 | 1,17 |
| 50 | Journal of Entomological Research | 2 | 0,21 |
| 51 | Journal of Insect Science | 2 | 1,22 |
| 52 | Journal of the Faculty of Agriculture, Kyushu University | 2 | 0,27 |
| 53 | Journal of Thermal Biology | 2 | 2,36 |
| 54 | Molecular Biology And Evolution | 2 | 16,24 |
| 55 | Nature Communications | 2 | 14,92 |
| 56 | Neotropical Entomology | 2 | 1,33 |
| 57 | Turkiye Entomoloji Dergisi | 2 | 0,57 |
| 58 | Academic Journal of Manufacturing Engineering | 1 | 0,32 |
| 59 | Acta Agriculturae Scandinavica A: Animal Sciences | 1 | 0,32 |
| 60 | Acta Biomaterialia | 1 | 8,95 |
| 61 | ACTA BRASILIENSIS | 1 | 0,00 |
| 62 | Acta Scientiarum | 1 | 0,62 |
| 63 | Agricultural and Forest Entomology | 1 | 1,89 |
| 64 | Annals of parasitology | 1 | 0,77 |
| 65 | Annals Of The Entomological Society Of America | 1 | 1,77 |
| 66 | Applied and Environmental Microbiology | 1 | 4,79 |
| 67 | Applied Ecology and Environmental Research | 1 | 0,71 |
| 68 | Arab Journal of Nuclear Science and Applications | 1 | 0,00 |
| 69 | Archives of Environmental Contamination and Toxicology | 1 | 1,86 |
| 70 | Archives of Insect Biochemistry and Physiology | 1 | 1,54 |
| 71 | Australasian Plant Pathology | 1 | 1,42 |
| 72 | Australian Journal of Botany | 1 | 1,39 |
| 73 | Australian Journal of Crop Science | 1 | 0,72 |
| 74 | Basic and Applied Ecology | 1 | 3,16 |
| 75 | Biodiversity Data Journal | 1 | 1,33 |
| 76 | Bioelectromagnetics | 1 | 2,28 |
| 77 | Bioinspiration and Biomimetics | 1 | 3,13 |
| 78 | Biology letters | 1 | 2,42 |
| 79 | Biology Open | 1 | 3,80 |
| 80 | BioMed Research International | 1 | 2,58 |
| 81 | Biophysics | 1 | 0,00 |
| 82 | BioSystems | 1 | 3,22 |
| 83 | Biosystems Engineering | 1 | 1,81 |
| 84 | BMC Evolutionary Biology | 1 | 3,06 |

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|---|---|---|---|
| 85 | BMC Genomics | 1 | 3,97 |
| 86 | Brain and Behavior | 1 | 2,53 |
| 87 | Bulgarian Journal of Agricultural Science | 1 | 0,69 |
| 88 | Bulletin of Insectology | 1 | 1,71 |
| 89 | Cell and Tissue Biology | 1 | 0,59 |
| 90 | Cell biochemistry | 1 | 4,28 |
| 91 | Cell Reports | 1 | 9,42 |
| 92 | Cellular and Molecular Life Sciences | 1 | 9,26 |
| 93 | Chemistry, Didactics, Ecology, Metrology | 1 | 0,00 |
| 94 | Chilean Journal of Agricultural & Animal Sciences | 1 | 0,47 |
| 95 | Chimia | 1 | 0,83 |
| 96 | Comptes Rendus de L'Academie Bulgare des Sciences | 1 | 0,34 |
| 97 | Conservation Physiology | 1 | 3,08 |
| 98 | Cryobiology | 1 | 2,05 |
| 99 | Developmental and Comparative Immunology | 1 | 3,19 |
| 100 | Diversity-Basel | 1 | 2,05 |
| 101 | Ecological Applications | 1 | 4,25 |
| 102 | Ecological Entomology | 1 | 1,85 |
| 103 | Ecosphere | 1 | 2,88 |
| 104 | eLife | 1 | 7,08 |
| 105 | ENTOMOLOGIA GENERALIS | 1 | 5,63 |
| 106 | ENTOMOLOGY AND APPLIED SCIENCE LETTERS | 1 | 1,91 |
| 107 | Environment International | 1 | 7,58 |
| 108 | Environmental Research | 1 | 5,03 |
| 109 | Environmental Sciences Europe | 1 | 5,39 |
| 110 | Environmental Toxicology and Chemistry | 1 | 3,18 |
| 111 | Epidenomes | 1 | 4,11 |
| 112 | Ethology Ecology and Evolution | 1 | 1,58 |
| 113 | European Journal of Neuroscience | 1 | 3,39 |
| 114 | Fresenius Environmental Bulletin | 1 | 0,55 |
| 115 | Frontiers in Cell and Developmental Biology | 1 | 5,19 |
| 116 | Frontiers in Physiology | 1 | 4,13 |
| 117 | Frontiers in Plant Science | 1 | 4,30 |
| 118 | Genes and Genomics | 1 | 1,19 |
| 119 | Genes, Brain and Behavior | 1 | 3,45 |
| 120 | Genetics and Molecular Biology | 1 | 1,88 |
| 121 | Heliyon | 1 | 1,85 |
| 122 | Hormones and Behavior | 1 | 4,45 |
| 123 | IEEE Access | 1 | 3,37 |
| 124 | IEEE Sensors Journal | 1 | 3,08 |
| 125 | IEEE Systems Journal | 1 | 5,28 |
| 126 | Indian Journal of Experimental Biology | 1 | 0,78 |
| 127 | Insect Science | 1 | 2,79 |
| 128 | Integrated Environmental Assessment and Management | 1 | 3,44 |
| 129 | International Journal of Advanced Robotic Systems | 1 | 1,48 |

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|---|---|---|---|
| 130 | International Journal of Agricultural and Statistical Sciences | 1 | 0,28 |
| 131 | International Journal of Cloud Computing | 1 | 0,43 |
| 132 | International Journal of Communication Systems | 1 | 1,32 |
| 133 | International Journal of Comparative Psychology | 1 | 0,85 |
| 134 | International Journal of Environmental Research | 1 | 0,40 |
| 135 | International Journal of Intelligent Engineering and Systems | 1 | 0,20 |
| 136 | International Journal of Wireless and Mobile Computing | 1 | 0,50 |
| 137 | International Transaction Journal Of Engineering Management & Applied Sciences & Technologies | 1 | 0,00 |
| 138 | iScience | 1 | 4,57 |
| 139 | JActa Fytotechnica et Zootechnica | 1 | 0,00 |
| 140 | Journal of Agricultural Science and Technology | 1 | 0,90 |
| 141 | Journal Of Agricultural Sciences-Tarim Bilimleri Dergisi | 1 | 0,40 |
| 142 | Journal of Ambient Intelligence and Humanized Computing | 1 | 4,81 |
| 143 | Journal of Animal Ecology | 1 | 4,55 |
| 144 | Journal of Applied Ecology | 1 | 6,53 |
| 145 | Journal of Biological Rhythms | 1 | 3,24 |
| 146 | Journal of Biosciences | 1 | 1,65 |
| 147 | Journal of Chemical Ecology | 1 | 2,12 |
| 148 | Journal of Comparative Physiology | 1 | 1,97 |
| 149 | Journal of Comparative Psychology | 1 | 1,77 |
| 150 | Journal of Enzyme Inhibition and Medicinal Chemistry | 1 | 4,31 |
| 151 | Journal of Evolutionary Biology | 1 | 2,72 |
| 152 | Journal of Green Engineering | 1 | 1,05 |
| 153 | Journal of Hazardous Materials | 1 | 9,04 |
| 154 | Journal of Insect Physiology | 1 | 2,51 |
| 155 | Journal of Invertebrate Pathology | 1 | 0,99 |
| 156 | Journal of Mathematical Biology | 1 | 1,94 |
| 157 | Journal of Proteome Research | 1 | 4,07 |
| 158 | Journal of the Kansas Entomological Society | 1 | 0,22 |
| 159 | Journal of the Mechanics and Physics of Solids | 1 | 5,00 |
| 160 | Journal of the Saudi Society of Agricultural Sciences | 1 | 6,46 |
| 161 | Journal of Theoretical and Applied Mechanics | 1 | 0,83 |
| 162 | Journal of Thermal Engineering | 1 | 0,71 |
| 163 | Jove-Journal Of Visualized Experiments | 1 | 1,40 |
| 164 | Letters in Applied Microbiology | 1 | 2,17 |
| 165 | Materials Research Express | 1 | 1,61 |
| 166 | Microbial Ecology | 1 | 3,36 |
| 167 | Microbial Pathogenesis | 1 | 2,91 |
| 168 | Microbiological Research | 1 | 5,42 |
| 169 | Microorganisms | 1 | 4,17 |
| 170 | Microscopy Research and Technique | 1 | 2,12 |
| 171 | Molecular and Cellular Probes | 1 | 1,87 |
| 172 | Molecular and Cellular Proteomics | 1 | 5,91 |
| 173 | Molecular Ecology | 1 | 6,19 |

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| 174 | mSystems | 1 | 5,85 |
| 175 | Nature Sustainability | 1 | 9,65 |
| 176 | Neural Computing and Applications | 1 | 4,77 |
| 177 | Neurobiology of Learning and Memory | 1 | 3,24 |
| 178 | Oecologia | 1 | 2,65 |
| 179 | Online Journal of Animal and Feed Research | 1 | 1,53 |
| 180 | OnLine Journal of Biological Sciences | 1 | 0,45 |
| 181 | Pacific Science | 1 | 0,82 |
| 182 | Pakistan Journal of Zoology | 1 | 0,92 |
| 183 | Palynology | 1 | 1,33 |
| 184 | Peer-to-Peer Networking and Applications | 1 | 2,79 |
| 185 | Pesticide Research Journal | 1 | 1,10 |
| 186 | PLoS Genetics | 1 | 5,91 |
| 187 | Polish Journal of Veterinary Sciences | 1 | 0,52 |
| 188 | Revista Chilena de Historia Natural | 1 | 1,41 |
| 189 | Revista Ciencia Agronomica | 1 | 0,50 |
| 190 | Revista Mexicana de Ciencias Pecuarias | 1 | 1,33 |
| 191 | Royal Society Open Science | 1 | 2,96 |
| 192 | Science | 1 | 41,85 |
| 193 | Science of the Total Environment | 1 | 7,96 |
| 194 | Semina: Ciencias Agrarias | 1 | 0,50 |
| 195 | SIAM Journal on Applied Mathematic | 1 | 1,55 |
| 196 | Soft Matter | 1 | 3,40 |
| 197 | Theoretical Population Biology | 1 | 1,26 |
| 198 | Urban Ecosystems | 1 | 2,55 |
| 199 | Veterinaria Y Zootecnia | 1 | 0,47 |
| 200 | Viruses | 1 | 5,05 |
| 201 | Vision Research | 1 | 2,61 |

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