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Aerobiology over Antarctica – a new initiative for atmospheric ecology

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1 **Aerobiology over Antarctica – a new initiative for atmospheric ecology**

2

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39

40 **Conflict of interest**

41 The authors declare no competing financial interests regarding this manuscript.

42

43

Provisional

44 **Abstract**

45 The role of aerial dispersal in shaping patterns of biodiversity remains poorly understood, mainly
46 due to a lack of coordinated efforts in gathering data at appropriate temporal and spatial scales. It
47 has been long known that the rate of dispersal to an ecosystem can significantly influence ecosystem
48 dynamics, and that aerial transport has been identified as an important source of biological input to
49 remote locations. With the considerable effort devoted in recent decades to understanding
50 atmospheric circulation in the south polar region, a unique opportunity has emerged to investigate
51 the atmospheric ecology of Antarctica, from local to continental scales. This concept note identifies
52 key questions in Antarctic microbial biogeography and the need for standardized sampling and
53 analysis protocols to address such questions. A consortium of polar aerobiologists is established to
54 bring together researchers with a common interest in the airborne dispersion of microbes and other
55 propagules in the Antarctic, with opportunities for comparative studies in the Arctic.

56
57 **Introduction**

58 Aerial dispersal plays an essential role in shaping patterns of biodiversity (Womack et al, 2010).
59 However, the ability of atmospheric ecology to help understand large scale patterns of biodiversity
60 remains limited, mainly due to a lack of coordinated efforts in gathering data at appropriate
61 temporal and spatial scales (Fig. 1). It has been long known that the rate of dispersal to an
62 ecosystem can significantly influence ecosystem dynamics; indeed, aerial transport has been
63 identified as an important source of biological input to remote locations (e.g., Pearce et al, 2010).
64 With the considerable effort devoted in recent decades to understanding Antarctic atmospheric
65 dynamics, we believe a unique opportunity has emerged to investigate atmospheric ecology from
66 regional to continental scales.

67 Despite the acknowledged importance of airborne microorganisms (including microscopic spores
68 and other propagules) (Fierer et al, 2008), most aerobiological studies have consistently failed to
69 consider the stability and viability of wind-borne microorganisms in the aerial environment. Whilst it
70 is assumed that potential colonists arrive continually from the atmosphere, for example, linked to
71 precipitation and wind-blown debris, the often extreme and selective nature of the atmospheric
72 environment is likely to limit the viability of the material transported to an unknown extent. With
73 evolution, extinction and colonization driving microbial biodiversity patterns, aerial dispersal
74 becomes intimately linked with eco-evolutionary dynamics across terrestrial, freshwater and marine
75 environments. Consequently, knowledge of the rates of airborne input, survival of the imposed
76 stresses of the transfer process, and viability on arrival, is essential for understanding ecosystem
77 stability and resilience.

78 Aerial biodiversity studies carried out to date have generally been based on single-site investigations
79 over limited time periods, providing 'snapshot' information on the abundance, distribution and
80 diversity of microorganisms found in specific aerial environments (e.g., Pearce et al, 2010; Fig. 2).
81 Although these have confirmed the magnitude of aerial dispersal, they have failed to address its
82 influence on ecosystem stability and resilience, only providing qualitative data in this regard.

83 A changing climate leads to changes in the frequency, intensity, spatial extent, duration, and timing
84 of extreme weather and climate events, and can result in unprecedented extreme weather and
85 climate events (IPCC 2012), so understanding the direct link between weather conditions and
86 biological dispersal is essential to determine the rate of climate-driven ecological change worldwide.
87 Here, we present a suggested methodology intended to gather wide ranging metadata relevant to
88 aerial ecology at representative temporal and spatial scales. The methodological approach discussed
89 here, and agreed by the pan-Antarctic initiative 'Aerobiology over Antarctica', provides a series of
90 sample handling guidelines and metadata characteristics required to ensure pan-Antarctic and

91 worldwide sampling consistency, and represents the first-ever coordinated effort to provide a
92 dynamic global map of aerobiological transport.

93

94 *The 'Aerobiology over Antarctica' consortium*

95 With recent agreement to co-ordinate weather and climate monitoring at the XIth Scientific
96 Committee on Antarctic Research (SCAR) symposium - Life in Antarctica: Boundaries and Gradients
97 in a Changing Environment, Barcelona, 15-18th July 2013, the necessary foundation exists to enable
98 establishment of a pan-Antarctic sampling initiative. For the first time, this initiative encompasses a
99 co-ordinated program to produce (i) a global dataset on aerobiological diversity and (ii)
100 contextualized environmental data aimed at clarifying the relationship between aerial biodiversity
101 and terrestrial ecosystem stability. At the XXXIIIth SCAR Open Science Conference, Auckland, New
102 Zealand, 23rd August – 3rd September 2014, a workshop was held to discuss the structure, sampling,
103 and environmental data recording methodologies, and common approaches to data analyses that
104 would be fundamental to the success of such a program, and would render it technically feasible
105 while also minimising costs. Aerobiological samplers are relatively light and easy to install, monitor
106 and use, with minimal power requirements. Furthermore, it is only relatively recently that the
107 logistic potential has existed to launch a co-ordinated continental (Antarctic) or even global field
108 sampling campaign. The analytical technology required for such an undertaking has only become
109 widely available with the advent of high-throughput DNA sequencing. This has allowed a departure
110 away from reliance solely on the more traditional culture-based microbiological approaches,
111 permitting a systematic analysis of the diversity of marker gene sequences and generating data that
112 are amenable to rigorous statistical analysis.

113 Initial discussions on program development have involved participants representing 27 institutions
114 from 19 countries. The key challenge in this type of study, as for many studies in microbial ecology, is

115 that the abundance and composition of airborne communities is variable across time and space. This
116 means that a large area (global or pan-continental) aerobiological sampling initiative could be
117 compromised by the specific methods selected and the techniques used in different regions. To
118 overcome such challenges, we propose the use of standardized minimal air collection and sample
119 processing methodologies and statistical analyses, in order to identify and detect patterns in
120 aerobiological datasets obtained from a wide variety of sources and approaches.

121

122 *The atmosphere as habitat for microorganisms*

123 Viable atmospheric biota are often assumed to be dormant and in a cryptobiotic state, with active
124 metabolism impossible in these harsh dry, low nutrient, high irradiance growth conditions. Although
125 a number of studies challenge this paradigm (e.g. Sattler et al. 2001), atmospheric diversity and
126 ecology, and the critical microbial biomass required to colonize a particular environment and
127 effectively influence its ecological dynamics, remain unexplored. Antarctic studies to date seem to
128 suggest a strong relationship between aerial propagules and terrestrial flora (e.g. Hughes et al.
129 2004), highlighting the need to understand the nature and direction of these interactions.

130 Airborne microorganisms may play an important role in the global climate system by absorbing or
131 reflecting incoming sunlight, acting as cloud condensation nuclei or serving as ice nucleating particles
132 (see e.g., Mohler et al, 2007). Their metabolic reactions can alter the atmosphere's chemical
133 composition, including the production of carboxylic acids from common atmospheric compounds
134 (Amato et al, 2007). Using incubation of cloud water, a recent study highlighted the activity of
135 microorganisms as an alternative route in photochemistry and showed that they significantly alter
136 OH radical production via H₂O₂ degradation (Vaithilingom et al, 2013). In addition, once deposited on
137 snow, microbes may participate in and alter other biogeochemical cycles (e.g., Maccario et al, 2014).

138

139 *Biogeography of microorganisms*

140 While progress has been made in microbial biogeography with respect to categorizing the observed
141 microbial distribution in space and time (Martiny et al. 2006; Fierer, N. & Jackson, R. B. 2006;
142 O'Malley, M. A. 2007; Wilkinson, et al. 2012; King, A. J. et al. 2010; Lutz, S. et al. 2015 a, b), especially
143 for single species, we are still far from a complete understanding of the factors that control the
144 process. Yet, invasions by non-indigenous species have been identified amongst the greatest threats
145 to global biodiversity (Litchman, 2010) particularly in response to disturbance and this, in turn, can
146 affect ecosystem structure and function. There is also the issue of airborne human disease
147 outbreaks. One of the mechanisms to explain microbial biogeographic patterns is dispersal.
148 However, there are limited empirical observations to support the role and significance of air
149 dispersal that has been hypothesized in microbial biogeography. Aerobiology, and concurrent
150 research on local features en route of the air mass transport, is therefore important to provide
151 evidence of connections between the airborne microbial assemblages and biota in surface habitats.
152 As a consequence, there are still major gaps in our understanding of airborne microbial diversity and
153 distribution, and the potential influence of airborne strains on the underlying terrestrial
154 environment (Womack, 2010).

155

156 *Using Antarctica to investigate global microbial dispersal*

157 Antarctica is the most remote continent on Earth. Its isolation from the rest of the world through the
158 Southern Ocean's Antarctic circumpolar current and the atmospheric circumpolar vortex and 'west
159 wind drift' makes it particularly well suited for studies involving the aerial transport and survival of
160 microorganisms and other transported biota (Siegert et al, 2008). Previous studies (see e.g., Vincent,
161 2000) have discussed the frequent transfer of biological material to Antarctica by atmospheric
162 processes. However, little is known about the contribution of bioaerosol transport to the microbial

163 ecology of isolated systems on the Antarctic continent (Bottos et al, 2014). Data on long-distance
164 dispersal of airborne organisms by trade winds are limited for microbes dispersed into the Antarctic
165 environment (Hughes et al, 2004), as well as data on their viability, duration of suspension and
166 gravitational settlement. In addition, the origin and maintenance of endemic populations in isolated
167 regions implicitly must be indicative of a (low) rate of airborne exogenous inputs (i.e. a lack of
168 genetic homogenisation), although this has proven hard to confirm and, rather, distinct bio-aerosol
169 communities are often reported (e.g. Bottos et al, 2014). On the other hand, the high percentage of
170 biological provinces endemic to specific Antarctic areas may be an artefact caused by the lack of
171 continental-wide biodiversity surveys. Ultimately, its level of isolation, combined with an extreme
172 environment able to challenge the viability of long-range colonists, and the presence of widely
173 distributed groups (such as cyanobacteria, diatoms, ciliates, rotifers, crustaceans in freshwater
174 systems, and terrestrial invertebrates, bryophytes and lichens), many of which are typified by
175 cryptobiotic life stages and/or resistant dispersing propagules, makes the Antarctic an ideal platform
176 for this type of study. Antarctic environments are also among the least human-modified terrestrial
177 ecosystems on earth, enabling accurate interpretation of patterns of genetic diversification or
178 dispersal. These relatively simple terrestrial ecosystems allow ecological communities to be
179 surveyed in unprecedented detail, to an extent not feasible in more species-rich ecosystems. Snow
180 and ice have largely low levels of microbial life compared to marine or terrestrial environments. This
181 makes interpretation of data collected on Antarctic ice-free 'islands' more straightforward, i.e. the
182 background contamination between propagule source and those collected/detected at the
183 destination is greatly reduced compared to other parts of the planet.

184

185 **Methods**

186

187 A balance needs to be struck between the main aim of the consortium – to encourage the collecting
188 of metadata of as wider variety of types as is possible and also a practical suggestion for those who
189 seek guidance on methodology. A suggested method is summarized in Table 1, but it should be
190 noted that this is a suggestion and not a recommendation or consensus.

191

192 *Sampling* The results generated by aerobiological sampling depend heavily upon the sampling
193 method used. This can be either passive, allowing particles to collect through natural processes such
194 as air movement or gravity, or active, where large volumes of air are passed over or through a
195 means of entrapment (reviewed by Griffin et al. 2011). Methods range through simple drop plates
196 (which can be augmented by different selective media), suction onto dry or gelatine filters (either via
197 commercial aerobiological sampling equipment or simple pump systems), to the many different
198 impactor approaches (i.e., solid and liquid). Whilst one outcome of the Auckland workshop was a
199 recommendation for active accumulation onto a 0.2 µm 47 mm diameter polycarbonate filter, it is
200 clear that a variety of different sample methods would also be useful to assess sampling bias. The
201 ideal approach depends on whether the information needed is qualitative or quantitative, highly
202 specific or of a general nature, highly localized or over a broader landscape. It also depends on
203 funding in the researcher's country, logistic field opportunities, and on ground support. The
204 combined strengths of selective culture, multiplexed molecular methods, high-throughput
205 sequencing and new instrumentation are improving our ability to simultaneously detect a wide
206 variety of organisms against a complex and variable natural background. Despite clear differences on
207 the merits and limitations of different methods, there is no clear consensus on an ideal approach.
208 The more traditional methods, including culturing on selective media, continue to have utility as
209 they demonstrate viability of those cells amenable to culture, although as is well known not all viable
210 cells will grow. As technology advances in microbial ecology, so do the approaches available, for

211 example the application of real-time quantitative PCR (e.g., Smith et al. 2012) and metagenomic
212 analyses (e.g., Smith et al. 2013).

213

214 *Sampling platforms* There are a very wide variety of possible sampling platforms, from ground level
215 to high altitude, and from the individual scientist with a single plate to an aircraft or weather balloon
216 custom-fitted to collect air samples. Sampler positioning will influence the material that is collected,
217 as will the existence of local obstructions (i.e. topography) which might induce turbulence. For the
218 project wider data collection, variety is the key. Different projects will use different methodologies,
219 and it is the diversity of these different sampling platforms which will add strength to the data
220 collected. It is anticipated, though, that most might be sampled close to weather stations, and below
221 c. 5 m in altitude, for practical reasons. Where possible, care should be taken to try and account for
222 transfer of the biota between the near-surface atmosphere and the boundary layer just above the
223 ground surface. For instance, rather than sampling at a single height, important relevant information
224 would be generated by deploying paired samplers at approximately 3 m (for capturing long-range
225 dispersed microbiota) and at c. 0.3 m to detect those near to the event of landing.

226 *Scale of sampling* It is well documented that air samples collected from different locations may differ
227 with respect to the relative abundances of specific bacterial and fungal groups (e.g., Marshall 1996).
228 The information obtained through this initiative at the Antarctic continental level, including inflow
229 and outflow, will provide a robust foundation for eventual scaling up to the global level. Sampling
230 will inevitably include air masses that move into, around, and away from the continent; however,
231 individual studies might range from a single sample or small numbers of samples taken every few
232 metres, to sampling locations separated by hundreds of kilometres, depending on the nature of the
233 particular project. Coverage will be the key here, and analyses at different spatial scales will enhance
234 the quality of the data. The advantage of using DNA as a target molecule for biodiversity studies is
235 that it does not exclude different target groups: viruses, prokaryotes and eukaryotes.

236 *Duration of sampling* The time spent sampling is important for aerobiology, as propagules can be
237 assayed per litre of air. Sample times may range between a few seconds and a few years. Longer
238 sampling times should yield higher numbers of propagules. Fierer et al. (2008) demonstrated short-
239 term temporal variability in airborne bacterial and fungal populations. Their results suggested that
240 outdoor air could harbour similar types of bacteria, regardless of location, and that the short-term
241 temporal variability in airborne bacterial assemblages can be very large. For particularly low biomass
242 systems such as the Antarctic, it is expected that large volumes might be needed as propagule
243 density is typically several orders of magnitude lower than that typical over lower latitude continents
244 (Burrows et al. 2009). This requires a trade-off between sampling periods short enough to avoid
245 desiccation or damage to samples against long enough to sample sufficient biomass to give
246 meaningful data. To this end, Durand et al. (2001) investigated the effect of sample time on the
247 culturability of airborne fungi and bacteria sampled by filtration, reporting no loss in viability. There
248 are already studies of this type, and it is anticipated that a variety of approaches will enhance the
249 quality of the data.

250 *Sample integrity* Aerobiological studies have sometimes been hampered at the publication stage by
251 sample integrity. In an ideal world, the aspiration would be to use completely sterile sampling
252 equipment, avoid any human contact, and process all material in a dedicated and certified clean
253 laboratory. However, this is not always practical, especially under Antarctic field conditions.
254 Attempts can be made to minimize contamination of sample material, such as the use of sterile
255 materials, stringent procedural negative and positive controls, the use of barrier type personal
256 protective equipment, and by returning sealed samples under sterile conditions for processing in
257 more controlled laboratories in researchers' home countries. However, further analyses of the data
258 obtained might increase understanding of the nature of in-process contamination risks. Indeed, such
259 contaminants (i.e. microorganisms brought in as a consequence of researcher activity) are part of
260 the contemporary Antarctic environment and so may, themselves, be considered a valid research

261 target and an important part of the analyses carried out (Pearce et al.2010; Hughes et al. 2011;
262 Cowan et al. 2011).

263 *Method of analysis* All methods in microbiology, without exception, are subject to bias and
264 limitations, and this means that a polyphasic approach is often the only way to ensure the reliability
265 of results. The most frequently used aerobiological techniques are culture, microscopy and DNA
266 extraction followed by high-throughput sequencing. For the studies we propose, a polyphasic
267 approach is indeed optimal; however, some co-ordination would be helpful in the final analysis, such
268 as the selection of the same DNA extraction methodologies and homologous gene regions for high-
269 throughput sequencing.

270

271 **Contextual data**

272

273 *Meteorological data* In order to make sense of the aerobiological diversity, it is important to collect
274 environmental context data. In combination with backtrack analyses, researchers also need to
275 consider the conditions the air mass has or will experience *en route* between two regions, not just
276 those at the 'landing site', as these will determine survival of transfer. Collaboration with current
277 platforms, such as the MCM TON (McMurdo Terrestrial Observation Network - these networks are
278 being designed to monitor key physical and biological processes associated with changing
279 ecosystems across regional to continental spatial scales by facilitating coordination and
280 comparability of measurements) and ANTOS (SCAR Antarctic Nearshore and Terrestrial Observing
281 System) initiatives, would help generate a standard suite of environmental parameters.

282 Relevant parameters include wind speed (instantaneous and over time), direction, fetch, humidity,
283 precipitation, barometric pressure (Woo et al. (2013), light and ultra-violet intensity, storm proximity
284 (Marshall et al. 1996), location (for proximity to potential terrestrial and marine inputs),

285 temperature, composition (e.g. moisture, salt content, dust inputs), and chemistry (e.g. ozone, ice
286 nucleating agents).

287

288 *Modelling* Different numerical models have been used in aerobiology over a range of applications,
289 including pollen dispersal (and allergy susceptibility), species invasions, spread of diseases and air
290 pollution (see e.g., Garcia-Mozo et al., 2009). These models represent useful tools to test current
291 ecological hypothesis. For example, data from the Antarctic Mesoscale Prediction System (AMPS)
292 and the application of NOAA HySPLIT system could be used to create back trajectories over the
293 Antarctic continent, indicating the sources of particular air masses and, potentially, their contained
294 biota. The co-ordinated sampling approach outlined here would provide observational data for use
295 in modelling studies (including community compositions and species distributions), particularly if
296 combined with meteorological data (Westbrook, 2010). Fitted models, including Structural Equation
297 Models (SEM), Generalized Linear Mixed Models (GLMMs), and Simultaneous Autoregressive
298 Models (SARs) can in addition take into account spatial autocorrelation.

299

300 *Reproducibility* Most studies completed to date have inevitably involved one-off or opportunistic
301 sampling. The data generated through this initiative, and classified in the form of metadata, might
302 allow the reproducibility of sampling to be assessed. It is essential to know whether the observations
303 are random, or whether patterns are apparent in the observations that can be attributed to specific
304 environmental characteristics. Previous researchers (e.g., Smith et al, 2012; 2013) have addressed
305 seasonal variability in airborne bacterial communities. They examined seasonal shifts in microbial
306 abundance and viability, and independently observed seasonality corresponding to highest
307 concentrations of bioaerosols.

308

309 *Data management* The datasets likely to be generated by this initiative are large but not necessarily
310 complex. A number of data management initiatives are already underway through SCAR, and would
311 be appropriate to utilise here. For example, the Microbial Antarctic Resource System (mARS) for
312 sequence data (using MIMARK environmental data format guidelines), the Polar and Alpine
313 Microbial Collection (KOPRI, Korea), the collection of polar cyanobacteria BCCM/ULC (Liege,
314 Belgium), the DNA repository for long term DNA storage (University of Waikato, New Zealand), the
315 SCAR Antarctic Terrestrial Biodiversity Database (Australian Antarctic Data Centre), and the Antarctic
316 Plant Database (held at the British Antarctic Survey)."

317

318 *Next steps* To get involved, register your project with the consortium. We will develop and host a
319 metadata repository to identify ongoing and prospective studies, that can be used to suggest links
320 and collaborations that can lead to enhanced datasets. Registrants will have opportunity to become
321 contributors to a coordination workshop to analyse and develop the next stage of implementation of
322 the project 'Gathering Antarctica large scale spatial and temporal airborne microbial samples to
323 understand the role of airborne input on continental Antarctic ecosystem function, its resilience and
324 stability'.

325

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328

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421 **Figure legends**

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423 **Figure 1. Distribution of aerobiological studies worldwide to date.** The map shows the
424 number of aerobiology studies published in English (and indexed in Scopus), as a measure of
425 the uneven and scattered distribution of aerobiological studies worldwide.

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427 **Figure 2. Distribution of aerobiological studies over Antarctica.** Data extracted from studies
428 indexed in ISI World of Science and those available to the authors but not indexed, published
429 between 1994 and 2014. A total of 12 studies were included. No studies prior 1994 were
430 available in ISI Web of Science. Circle diameter indicates the number of sites included per
431 study.

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Caption: Table 1. Summary of the proposed method and contextual data.	
Method	
<i>Sampling</i>	Active accumulation onto dry 0.2 µm 47 mm diameter sterile polycarbonate filters supported by a variety of different sampling methods to enhance the quality of the data.
<i>Sampling platforms</i>	Aim for 3 m above ground level to minimize local effects, whilst still being supported by a variety of different sample heights to enhance the quality of the data.
<i>Scale of sampling</i>	Target all microorganisms and biological material containing DNA. A minimum of three replicates per site and as wide coverage as is practical.
<i>Duration of sampling</i>	Sample a minimum of 24 h assay for biomass and extend as long as practical.
<i>Sample integrity</i>	Use best practice feasible for the field location in question. The essential component here is an accurate and detailed description of the methodology employed.
<i>Method of analysis</i>	Microscopy, culture and DNA extraction and analysis using high throughput sequencing. Here, for instance, we suggest the V3-V4 hypervariable region (Caporaso et al. 2012) for the simultaneous detection of bacterial and archaea, 18S and virus markers. We also suggest including shotgun metagenomic analysis which will cover all groups and functions. Some form of

	biomass quantification is desirable.
Contextual data	
<i>Meteorological data</i>	By collaborating with a multi-national continent-wide observing system ensure that sampling sites are congruent with environmental monitoring stations. This will provide a suite of parameters that can be used to clarify the links between airborne microbes and the associated physical environment.
<i>Modelling</i>	Use tested and contemporary models to clarify the relationship between airborne microbe biodiversity and associated environmental parameters.
<i>Reproducibility</i>	Repeat sampling at intervals throughout the year and in multiple years as logistic opportunity permits.
<i>Data management</i>	Adopt mARS and utilise other culture collection repositories.

Figure 1.JPEG

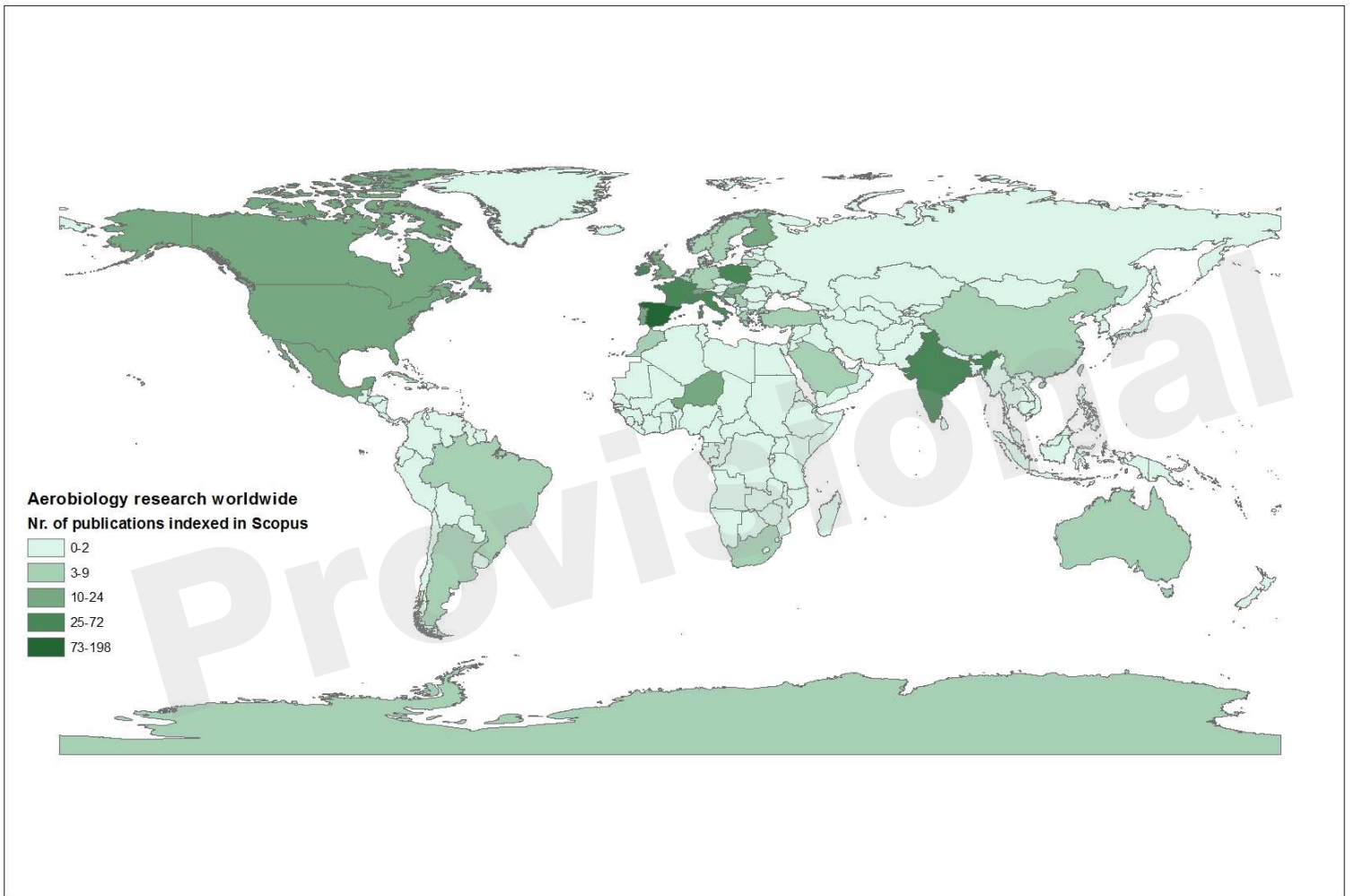


Figure 2.JPEG

