

Potential Distribution Map of *Culicoides insignis* (Diptera: Ceratopogonidae), Vector of Bluetongue Virus, in Northwestern Argentina

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Abstract

Culicoides insignis Lutz is incriminated as a vector of bluetongue virus (BTV) to ruminants in America. In South America, almost all countries have serological evidence of BTV infections, but only four outbreaks of the disease have been reported. Although clinical diseases have never been cited in Argentina, viral activity has been detected in cattle. In this study, we developed a potential distribution map of *Culicoides insignis* populations in northwestern Argentina using Maximum Entropy Modeling (Maxent). For the analyses, information regarding both data of specimen collections between 2003 and 2013, and climatic and environmental variables was used. Variables selection was based on the ecological relevance in relation to *Culicoides* spp. biology and distribution in the area. The best Maxent model according to the Jackknife test included 53 *C. insignis* presence records and precipitation of the warmest quarter, altitude, and precipitation of the wettest month. Accuracy was evaluated by the area under the curve (AUC = 0.97). These results provide an important analytical resource of high potential for both the development of suitable control strategies and the assessment of disease transmission risk in the region.

Key words: biting midges, vectors, potential distribution, Argentina

Females of *Culicoides* biting midges (Diptera: Nematocera: Ceratopogonidae) are hematophagous involved in the transmission of arbovirus, protozoa, and filarial nematodes that cause diseases both in humans and animals (Borkent and Spinelli 2007). At the global scale, the pathogenicity of the diseases transmitted by *Culicoides* spp. to humans is limited, for which the epidemiological relevance of biting midges has generally been related to animal health. Bluetongue virus (BTV) is the most important disease due to its wide geographical distribution, rapid spread, and significant economic impact (OIE 2008, Carpenter et al. 2013).

BTV is an infectious, noncontagious virus which affects both domestic and wild ruminants, causing hemorrhage and ulceration in the upper gastrointestinal tract as well as laminitis, coronitis, facial and neck edema, pulmonary edema, reproductive failures, and lameness (Mellor et al. 2009). At the present, 26 serotypes have been recognized worldwide (Maan et al. 2012), four of which are endemic

of North America, (MacLachlan and Guthrie 2010) and seven of Central America and The Caribbean (Mertens et al. 2005). In South America, the serological evidence of BTV has been reported in Ecuador, Peru, Chile, Guyana, Argentina, and Brazil, but it has only been isolated in the last three countries (Lager 2004, Clavijo et al. 2012, Legisa et al. 2014). In Argentina, the clinical aspects of the disease have never been reported, but the virus (serotype 4) has been isolated from cattle from Corrientes province, which represented the first isolation in the country (Gorchs et al. 2002, Lager 2004, Legisa et al. 2013).

Culicoides species described as competent vectors of BTV exhibit differences within continents. In Central America and The Caribbean, *Culicoides insignis* Lutz, *Culicoides filarifer* Hoffman, and *Culicoides pusillus* Lutz were reported (Greiner et al. 1992, Walton and Osburn 1992, Mo et al. 1994), whereas in South America Lager (2004) reported *C. insignis* as the most likely vector

species of BTV in Argentina. In general, weather conditions in South America are suitable for the development of *Culicoides* specimens, and thus, for BTV transmission (Homan et al. 1985, Lopez et al. 1985). Furthermore, in northwestern Argentina, *Culicoides paraensis* Goeldi and *C. insignis* are dominant species in terms of abundance of individuals (Veggiani Aybar et al. 2010, 2011, 2012). Other studies also reported *C. insignis* and *C. pusillus* as the main vectors in South America (Gibbs et al. 1983, Homan et al. 1990, Greiner et al. 1992, Gouveia et al. 2003, Felipe-Bauer et al. 2008, Legisa et al. 2013). BTV has been isolated from these species of *Culicoides* in Central America and The Caribbean, but not in South America, although the clinical signs and pathological to the disease it has been described in Brazil (OIE 2001, Balaro et al. 2014).

In South America, it has been pointed out that certain geographical features should be considered when analyzing the distribution of BTV, such as the geographical isolation of Chile from the rest of the countries by the Andes Mountains and the Atacama Desert (Legisa et al. 2014). The latter, which reaches the north of Argentina and the south of Bolivia, has been suggested as a barrier for BTV due to specific climate conditions which might not be suitable for *Culicoides* spp. life cycle (Puntel et al. 1998; Marcoppido et al. 2010, 2011; Legisa et al. 2013, 2014). Also, altitude has been associated with the absence of BTV detection in certain areas of Brazil, Colombia, and Venezuela (Homan et al. 1985, Gonzalez et al. 2000, Tomich 2007), with a negative correlation between altitude and the life cycle of the vector (Legisa et al. 2014). Although geographical barriers limit the distribution patterns of certain vector species, global climate change might also contribute to the spread of BTV, by mainly creating more suitable conditions for the spreading and reproduction of the vector (Wittmann and Baylis 2000). This situation highlights the importance of analyzing the importance of the knowledge regarding the presence, abundance and distribution of *Culicoides* species, especially *C. insignis*, in the region. Also, its potential vectorial capacity in BTV transmission needs to be further assessed.

Entomological studies conducted in northwestern Argentina determined both the presence and abundance of *Culicoides* spp. and the environmental factors influencing their spatio-temporal dynamics (Veggiani Aybar et al. 2010, 2012). However, being vectors of great sanitary importance, updates regarding their ecology and distribution at different scales in the context of climate change are of urgent need for the development of risk profiles of the diseases they transmit. In this sense, Maxent (Maximum entropy) is a widely used statistical modeling method for determining species distribution based on species presence data (Phillips et al. 2006). Maxent estimates the correlation between environmental or climatic variables and species presence, and although it should not be used to infer causative relationships or underlying mechanisms (Rogers 2006, Dormann et al. 2012), it can provide useful insights on current distributions when based on reliable ecological data (Dormann et al. 2012). The objective of the present study was to generate a potential distribution map of *Culicoides insignis* in northwestern Argentina using Maxent, which would allow assessing the potential risks in relation to BTV emergence in the region.

Materials and Methods

Specimens Collection and Processing

The present study was focused on *C. insignis* presence data. Each one of the 53 sites/localities was georeferenced, and they were situated in the subtropical mountainous rainforest also called Yungas

at the Salta (180,000 m²), Jujuy (100,000 m²), and Tucumán (780,000 m²) provinces.

Yungas ecoregion is distributed in northwestern Argentina and southern Bolivia over approximately 56,000 km². The mean temperature is comprised between 14 and 26°C and the annual rainfall between 900 and 2,500 mm. The vegetation extends across a large altitudinal gradient (400–2,300 masl). Four layers of vegetation exist within the forest (pedmont forest, mountain forest, mountain wood, and altitude grasses) (Malizia et al. 2012).

Collection of *Culicoides* species was performed between 2003 and 2013 using CDC light traps with ultraviolet light. A total of six traps per sampling site/locality were used, which were placed on the branches of trees at a height of 1.20 m above the ground at a distance of 100 m from one another. The traps remained active for two consecutive days from 17:00 p.m. to 08:00 a.m. of the following day, and they were placed in the localities selected according to altitudinal gradients, latitudes, water resources, and degrees of anthropic interventions (houses, crops, and breeding farms). Subsequently, specimens were separated and identified to the species level following Spinelli et al. (2005) taxonomical key.

Ecological Niche Modeling

Maxent software version 3.3.3k, a species distribution modeling tool based on maximum entropy (Phillips et al. 2006, Phillips and Dudik 2008), was used to estimate the distribution probability of *C. insignis*. This software requires only species presence data and environmental variables layers for the area of interest (Pearce and Boyce 2006). As a result, Maxent indicates the probability to find a given species, ranked from 0 (least suitable habitat) to 1 (most suitable habitat).

Nineteen bioclimatic variables (BIO1–BIO19) (Table 1) were used as potential predictors of *C. insignis* distribution. These variables were derived from WorldClim database (<http://www.worldclim.org/bioclim>), at a spatial resolution of 30 arc-seconds, and each cell is a square of approximately 1 km side (0.93 by 0.93 = 0.86 km²) (Hijmans et al. 2005).

For evaluating the model, 75% of *C. insignis* presence sites were used as training data, and the 25% left were used as testing points, in order to search for the statistical significance of the test. Model performance was assessed using a threshold independent method based on the area under the curve (AUC) of the Receiver Operating Characteristics curve. AUC takes values from 0 to 1, but robust models values rank from 0.7 to 0.9, while values above 0.9 characterize models with almost perfect discrimination (Fielding and Bell 1997, Pearce and Ferrier 2000). In this sense, an AUC score of 1 would represent perfect prediction, with zero omission (while AUC values equal to 0.5 would be expected from a random prediction). Also, the contribution of each explanatory variable to the overall model was evaluated with a jackknife test, in which the variables are successively omitted and then used in isolation to measure their relative and absolute contribution to the model. The most important variables are those which result in the highest training gains, or reduce it when left out of the model.

Results

Maxent Analysis

The pixels meet the restriction conditions imposed by the values of the environmental variables of the species known localities. Since the logistic output was used, with values ranging from 0 to 1, an output map with five ranges of presence probabilities was created:

Table 1. Codes and relative contributions of the variables to the Maxent model of *Culicoides insignis*

Code	Variable	% contribution	Permutación importance
BIO1	Annual mean temperature	2.5	0.6
BIO2	Mean diurnal range	0.7	7.9
BIO3	Isothermality	0	0
BIO4	Temperature seasonality	0	0
BIO5	Max temperature of warmest month	0	0
BIO6	Min temperature of coldest month	0.1	0.3
BIO7	Temperature annual range	0	0
BIO8	Mean temperature of wettest quarter	2.3	2.7
BIO9	Mean temperature of driest quarter	0.7	0.1
BIO10	Mean temperature of warmest quarter	1.7	0.2
BIO11	Mean temperature of coldest quarter	0.2	24.4
BIO12	Annual precipitation	0	0
BIO13	Precipitation of wettest month	13.8	1.2
BIO14	Precipitation of driest month	0	0
BIO15	Precipitation seasonality	3.9	0
BIO16	Precipitation of wettest quarter	0	0
BIO17	Precipitation of driest quarter	0.5	0.4
BIO18	Precipitation of warmest quarter	33.9	0.1
BIO19	Precipitation of coldest quarter	6.7	2.8
DEM	Digital elevation model	32.9	59.4

0–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1. The resulting map revealed that the most suitable areas for *C. insignis* were mainly restricted to Monteros, Famaillá, Yerba Buena, Río Chico, Lules, Simoca, Chicligasta, Tafí Viejo, and Cruz Alta departments in Tucumán province; Orán, General José de San Martín, Iruya, and Santa Victoria departments in Salta province; Ledesma, Santa Bárbara, San Pedro, and Valle Grande departments in Jujuy province; Río Hondo and Guasayán departments in Santiago del Estero province; and Santa Rosa department in Catamarca province (Fig. 1).

Model Evaluation and Predictor Variables Influence

The model presented an AUC of 0.97, with a SD of 0.02. The variables that contributed most to the model were: precipitation of the warmest quarter (BIO18), followed by altitude, represented through a Digital Elevation Model (DEM) and the precipitation of the wettest month (BIO13). Furthermore, precipitation of the driest month (BIO14), annual temperature range (BIO7), isothermality (BIO3), annual precipitation (BIO12), temperature seasonality (BIO4), maximum temperature of warmest month (BIO5), and precipitation of the wettest quarter (BIO16) did not contribute to the model (Table 1). Since these variables were estimated heuristically, the jackknife test was applied, indicating which variables were the most important in the model and more effectively forecasted *C. insignis* distribution. The resulting variable was the precipitation of warmest quarter (BIO18) (Fig. 2). The jackknife test gave a total training gain of 3.8 (Fig. 2, red bar at the bottom of the chart), and selected the precipitation of warmest quarter (BIO18) as the variable with both the highest gain (1.7, dark blue bar) when used alone and which generated the highest decreases (3.8) when omitted. Thus, precipitation of

warmest quarter was the variable that most accurately predicted *C. insignis* distribution in northwestern Argentina. On the other hand, DEM exhibited a training gain of 0.9 (dark blue bar) when used in isolation and caused a gain drop of 3.4 (light blue bar) when omitted. Precipitation of the driest quarter (BIO17) and isothermality (BIO3) were not useful for estimating *C. insignis* distribution when used in isolation.

AUC value based on the validation dataset was 0.97 whereas AUC value in relation to the initial, training datasets was 0.995 (Fig. 3).

Discussion

The potential distribution of *C. insignis* in northwestern Argentina forecasted by the model fitted very well in the north and south areas of Tucumán and Salta provinces, whereas in the central area of Salta and Jujuy provinces, the distribution was unbalanced. These biases might be the result of a lack of presence records for these areas, since one of the disadvantages of maximum entropy algorithm is that it tends to over-fit the distribution according to the introduced points (Phillips et al. 2006). Other studies demonstrated a deterioration of modeling predictive performance as sample size decreased (Peterson et al. 2004, Pearson et al. 2007), while accurate models produced with few records can also be found in the literature, maybe because ecologically specialized species with smaller geographic ranges and very low tolerance can be well represented with fewer presence records (Hernandez et al. 2006). Nevertheless, our records showed that *C. insignis* is restricted mainly to piedmont (400–700 masl) and montane forests (700–1,500 masl) in Yungas ecoregion, and a portion of Chaco Serrano and Semi-arid Chaco ecoregions.

Climatic variables such as temperature, precipitation, humidity, etc, influence the life cycle *Culicoides* spp. in many aspects, including survival, abundance, pathogen-vector interactions, and behavior and distribution (Mellor et al. 2000; Tabachnick 2010; Veggiani Aybar et al. 2010, 2011, 2012; Carrasco et al. 2014). In this study, precipitation of the warmest quarter (BIO18) and precipitation of the wettest month (BIO13) indicates that these variables could be the triggering or limiting factor in the potential new distributions of *C. insignis*.

The niche model also predicts that the area encompassing central-southwestern Tucumán provides the most suitable environment for *C. insignis*, while its probability of occurrence ranges from low to moderate in northeastern and central Salta, southeastern Jujuy and western Tucumán, pattern consistent with the previous knowledge of the distribution of this species in the study area (Veggiani Aybar et al. 2010, 2011, 2012). It is worth mentioning that some of these areas are currently being modified by anthropic activity; for example, the expansion of agricultural lands in northwestern Tucumán and northern Salta (Fundación ProYungas 2011). Therefore, these changes might trigger the expansion of the abundance and distribution range of *C. insignis*, and we recommend the incorporation of land use variables in future models.

The results of this work highlight the potential ecological requirements of *C. insignis* at a regional scale and predict its northernmost distribution edge. Despite the well-known limitations of Maxent predictions, they should be considered as a reliable source of information on *Culicoides* species autochthonous populations distribution in other areas, which, in turn, could be extrapolated to future climatic scenarios in order to quantify larger scale effects in the context of climate change. Finally, expanding the range of *C. insignis* occurrence data in the northeast of the country, as well as

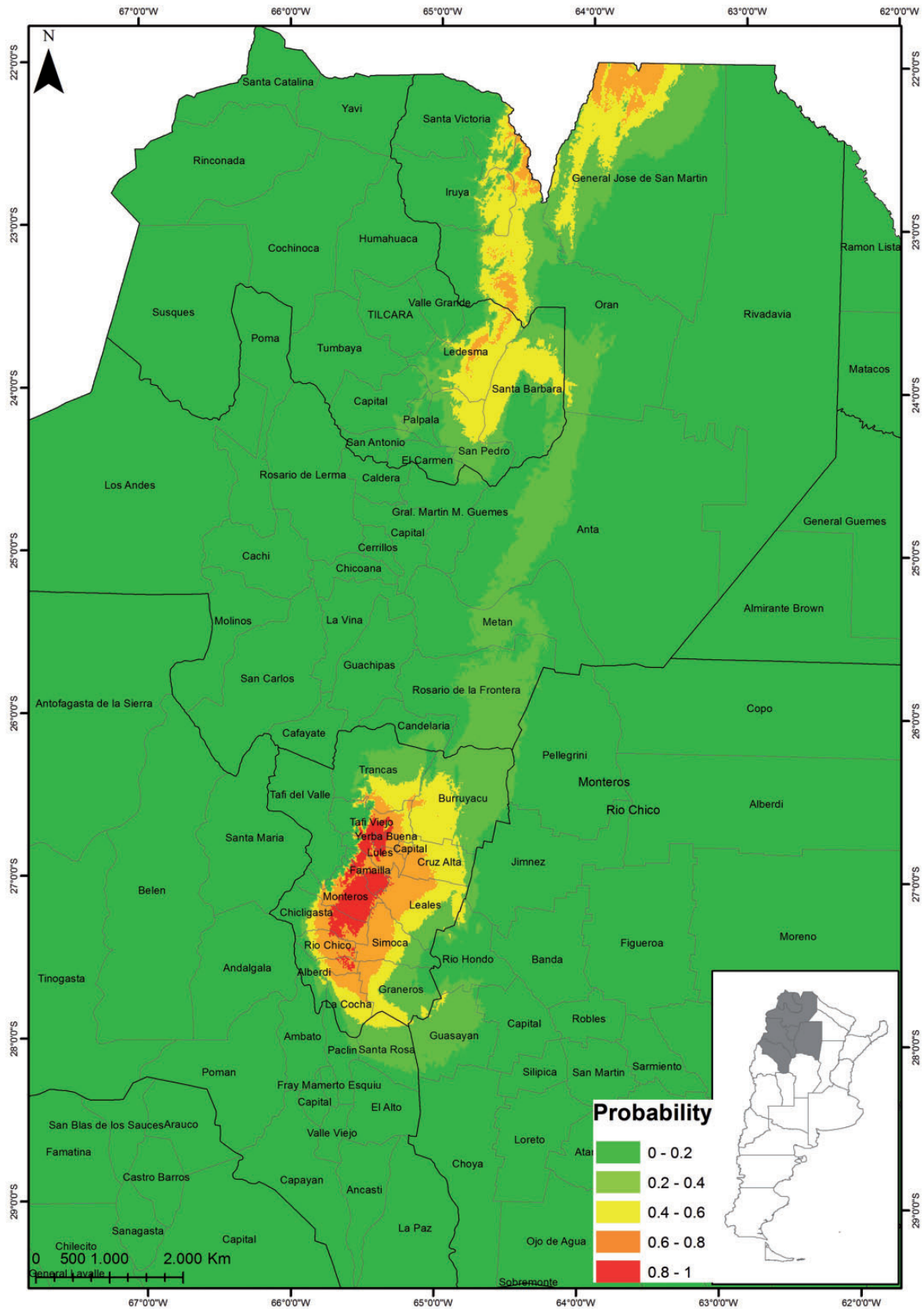


Fig. 1. Maxent map of the potential distribution of *Culicoides insignis* in northwestern Argentina. Low probability classes are represented in green (0–40%), intermediate probability (40–60%) in yellow, and high probability in orange (60–80%) and red (80–100%).

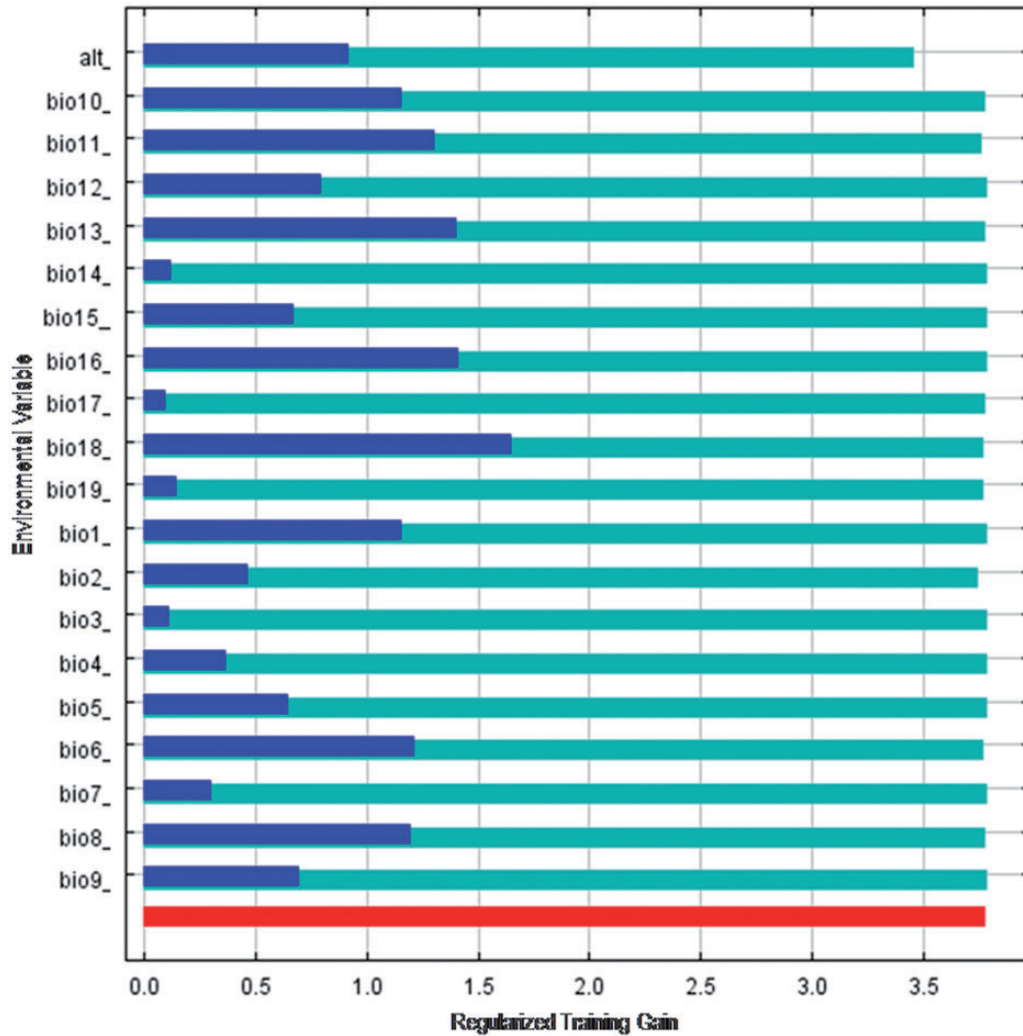


Fig. 2. Results of jackknife test of variable importance for *Culicoides insignis* populations. Blue bars show individual environmental variables importance relative to the red bar, which shows all environmental variables; light blue bar shows whether a variable gives information that is not given by the other variables, and dark blue bar shows whether a variable gives any useful information by itself. See code of the variables in Table 1.

the positive serotypes detected in ruminants in the region is of great importance, in order to establish updated ranges of distribution of both the vector and BTV in Argentina.

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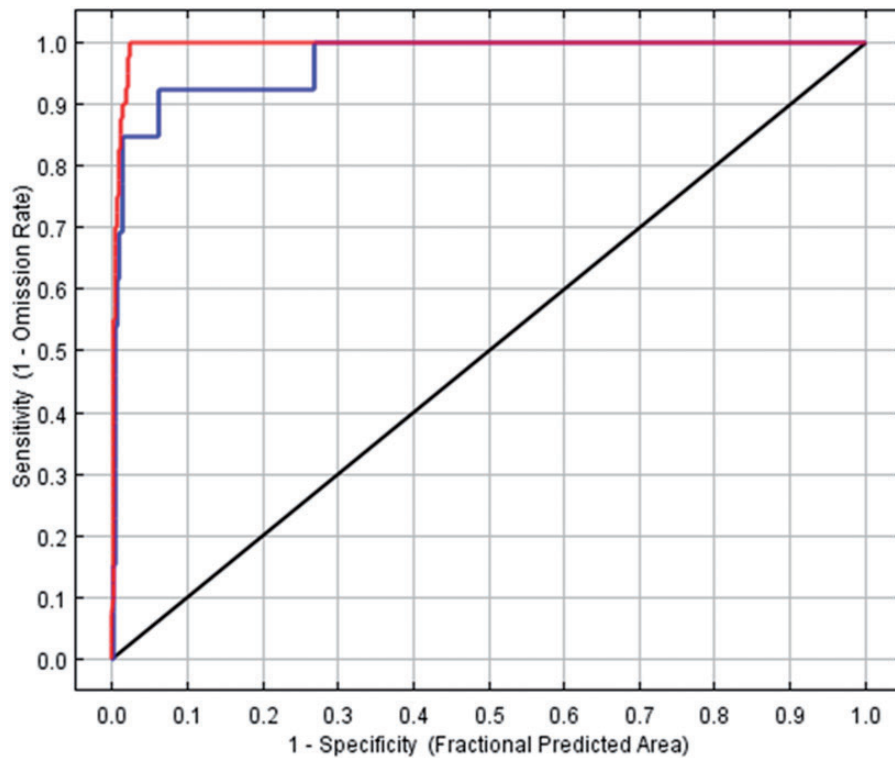


Fig. 3. Receiver Operating Characteristics (ROC) and Area Under the Curve (AUC) values for Maxent model of *Culicoides insignis*. Black line indicates random prediction (AUC = 0.5), the red line training data (AUC = 0.995), and the blue line the test data (AUC = 0.97).

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