EcoHealth
https://doi.org/10.1007/s10393-025-01770-6

EcoHealth

© 2025 The Author(s), under exclusive licence to Nature.Health.Global., Inc

Ecological Niche Modeling of *Acanthamoeba* in Türkiye

Hakan Kavur,[1] Gülşah Evyapan,[2] and Ozan Artun[1]

[1]*Karaisali Vocational School, Cukurova University, Karaisali, Adana, Turkey*
[2]*Faculty of Medicine, Department of Medical Biology, Yuzuncu Yil University, Van, Turkey*

Abstract: *Acanthamoeba*, is an opportunistic pathogenic organism with a global distribution and the potential to cause severe human infections. This study primarily aimed to identify the environmental factors influencing the distribution of *Acanthamoeba* by analyzing various bioclimatic and topographic variables, and to predict their potential current and future distribution under 2070 climate change scenarios using ecological niche modeling based on the MaxEnt algorithm. Niche modeling was performed on 20 water and 20 soil samples collected from hot springs, swimming pools, parks, and agricultural areas. The rates of positive water samples in Afyon and Kütahya were 70 and 50%, respectively. We detected 60 and 100% positive rates of soil samples collected in Afyon and Kütahya, respectively. Niche modeling incorporated 19 bioclimatic variables, with BIO3 (Isothermality), BIO4 (Temperature seasonality), BIO13 (Precipitation of the wettest month), and BIO15 (Precipitation seasonality) identified as the most influential predictors. The model showed high predictive performance, with AUC values of 0.991 and 0.977 for current and future projections, respectively. Results suggest a potential increase in *Acanthamoeba* distribution in future scenarios, especially in the southwestern region of Afyon and southern Kütahya. These findings highlight the importance of environmental monitoring and genotypic characterization of *Acanthamoeba* for public health risk assessment.

Keywords: *Acanthamoeba*, bioclimatic factors, ecological niche model, ArcGIS, MaxEnt

Introduction

Acanthamoeba is a ubiquitous, free-living protozoan. It can be found in diverse environments, including hot springs, soil, ice, air, heating ducts, fresh vegetables, bottled water, eyewash solutions, and the nasopharyngeal washes of asymptomatic individuals (Lorenzo-Morales et al. 2006). *Acanthamoeba* species have two life stages: trophozoites and cysts. Trophozoites are pathogenic due to their active feeding behavior. Cysts are highly resistant to environmental stresses such as starvation, anoxia, osmolarity changes, extreme pH, and temperature (Esboei et al. 2020).

Over the years, the environmental parameters supporting the presence of *Acanthamoeba* species and their significance for human health have been insufficiently emphasized (Booton et al. 2005; Kalra et al. 2020; Niederkorn, 2021; Pana et al. 2023). *Acanthamoeba* can cause infrequent but severe diseases. These include *Acanthamoeba keratitis* (AK), a sight-threatening corneal infection that

Publisher's Note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Correspondence to: Hakan Kavur, e-mail: hkavur@cu.edu.tr

Published online: 24 November 2025

predominantly affects contact lens wearers in developed countries (Carnt et al. 2018; Dos Santos et al. 2018). Granulomatous amebic encephalitis (GAE), a fatal infection of the central nervous system (CNS) and skin. In recent years, the incidence of GAE has been increasing among immunocompromised patients. AK often triggers a robust immune response, which can lead to severe inflammatory complications. These include scleritis (SIC), affecting about 20% of patients, and corneal stromal ring infiltration in 15% of patients (Bacon et al. 1993). One-third of affected patients experience permanent vision loss. Additionally, one in four patients requires corneal transplantation (Dart et al. 2009).

The distribution of *Acanthamoeba* species is global and influenced by local environmental and ecological conditions (Alves et al. 2012). In environmental sampling, the proportion of *Acanthamoeba* species has been detected at different rates, such as 33.3% in 48 soil samples from Iran (Basher et al. 2018). *Acanthamoeba* was detected at a high frequency [85% (136/160)] in environmental samples collected from dry soil, dust, wet debris, wet soil, and water (Meighani et al. 2018).

Increased *Acanthamoeba* numbers and extended water activity periods have been linked to a higher incidence during warmer seasons (Bouheraoua et al. 2014). Studies across various countries have found a high prevalence of *Acanthamoeba* species in human-related habitats (Coşkun et al. 2013; Esboei et al. 2020). Higher *Acanthamoeba* numbers and prolonged water activity have been associated with higher incidences of *Acanthamoeba* infections during warmer seasons (Bouheraoua et al. 2014).

Despite various genotyping studies conducted on *Acanthamoeba* species, there are a limited number of studies in Türkiye regarding the parameters affecting the presence and distribution of these species. Moreover, there were no previous studies related to *Acanthamoeba* epidemiology conducted in Afyon and Kütahya provinces. Due to their climates and the presence of thermal waters, Ankara and Sivas provinces exhibit similarities to Afyon and Kütahya provinces (Akbulut 2010). Therefore, a local survey conducted in Sivas, Türkiye, revealed that 22% (33/150) of tap water samples harbored free-living amoebae (FLA). Morphological and molecular analyses identified *Acanthamoeba castellanii* and *Acanthamoeba polyphaga*, both belonging to the potentially pathogenic T4 genotype, as well as *Hartmannella vermiformis* (Coşkun et al. 2013). In a related study from Ankara, 18 environmental *Acanthamoeba* isolates were genotyped, revealing the presence of T2, T3, T4, and T7 genotypes. Among these, T3 and T4 isolates demonstrated the highest pathogenic potential, showing temperature and osmotolerance, along with severe cytotoxic effects on corneal epithelial cells. These findings support the hypothesis that the high prevalence of genotype T4 in *Acanthamoeba keratitis* cases may be linked to its increased virulence and environmental abundance (Kılıç et al. 2004).

Molecular investigations conducted in western Türkiye have demonstrated a heterogeneous distribution of *Acanthamoeba* genotypes in environmental water sources, with T2, T4, T5, and T15 identified through 18S rRNA sequencing (Aykur and Dağcı, 2021). Notably, T4 remains predominant, consistent with its recognized pathogenic potential. Complementing these findings, clinical investigations into keratitis cases in Türkiye have reported the presence of both T4 and, for the first time, T5 genotypes, highlighting the emerging significance of T5 in human infections (Dirim Erdoğan and Aykur, 2022). These data underscore the importance of continuous monitoring of environmental reservoirs and clinical isolates in order to better understand the epidemiology and pathogenicity of *Acanthamoeba* genotypes circulating in Türkiye.

Geographic Information Systems (GIS) are software tools designed to capture, store, manipulate, analyze, manage, and visualize spatial and environmental data (Glass, 2001). GIS provides the infrastructure necessary for integrating and analyzing georeferenced datasets across various disciplines, including epidemiology. Ecological Niche Modeling (ENM) is a computational method that utilizes environmental variables to predict the potential geographic distribution of species based on their ecological requirements. Among ENM techniques, Maximum Entropy Modeling (MaxEnt) is widely adopted due to its capacity to efficiently handle presence only data and produce accurate predictions of species' suitable habitats (Elith et al. 2006). MaxEnt employs a machine learning algorithm grounded in solid mathematical foundations, offering a straightforward and robust framework for ecological modeling. Thus, while GIS facilitates spatial data management and analysis, ENM serves as the modeling approach for species distribution, with MaxEnt being a commonly used algorithm within ENM frameworks.

Using novel modeling techniques from MaxEnt, we investigated the current and projected (2070) distribution probability of *Acanthamoeba* species in Afyon-Kütahya under relevant bioclimatic conditions, a projected date when scientists will be able to assess whether conservation

strategies are sufficient. Tourism related to hot springs is prevalent in all of the localities we have selected and attracts many summer and winter visitors.

Materials and Methods

Research Region

In this study, 20 water and 20 soil samples were collected from Afyon and Kütahya provinces (Fig. 1) at different times in spring (March 2011 and May 2022). To minimize potential sampling bias, all sampling locations were selected using a random sampling approach, ensuring that the sites were spatially well distributed across different habitats and environmental conditions. Notably, Afyon and Kütahya are among the leading provinces in Türkiye for thermal spring tourism, which may influence the presence and distribution of *Acanthamoeba*. Although absence data were not available, MaxEnt is suitable for presence only data, and background points were used to represent the available environmental space (Raes and ter Steege, 2007).

The province of Afyon, located in the western region of Türkiye, is characterized by a continental climate. This climate is marked by cold and snowy winters, as well as hot and dry summers. The locality serves as the focal point of an agrarian region and boasts contemporary, adequately furnished lodgings and wellness facilities. The presence of mineral-rich waters, thermal springs, and indoor swimming pools attracts a significant number of visitors during the winter season. Kütahya, located in the western region of Türkiye, exhibits a climatic pattern similar to that of Afyon. Kütahya is renowned for its Turkish baths situated within the region. Despite these similarities, the provinces also show notable variations in certain environmental attributes.

Sampling, Culture Methods and DNA Sequence Analysis

The *Acanthamoeba* culture methods utilized have been previously detailed by Lorenzo-Morales et al. (2006). The methodology involved dissolving 2 g of soil samples in 20 ml of sterile distilled water. Subsequently, 150 µl of each sample were inoculated onto 2% non-nutrient agar plates previously seeded with heat-killed *E. coli*. After inoculation, the plates were incubated at 27 °C and examined daily for up to 14 days using an inverted microscope to detect *Acanthamoeba*.

PCR amplification targeting a 423–551 bp fragment of the 18S rRNA gene was performed using JDP1 (5′GCCCAGATCGTTTACCGGAA 3′) and JDP2 (5′TCTCACAGCTGCTAGGGGAGTCA 3′) primers (Schroeder et al. 2001). Thermocycling was programmed for 30 cycles of 60 s at 95 °C, 45 s at 55 °C, and 30 s at 72 °C. Products were visualized on 2% agarose gels stained with ethidium bromide under UV light.

The PCR products were sequenced using the BigDye Terminator kit on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). Sequences were analyzed via BLAST (www.ncbi.nlm.nih.gov/BLAST) and compared with GenBank entries. The phylogenetic analysis was constructed using the neighbor-joining (NJ) method and MEGA11 software. The distances were calculated using the Maximum Composite Likelihood method.



Figure 1. Study areas and points at which *Acanthamoeba* were collected.

Environmental Data

The present study employed ASTER GDEM Version 3 as the primary source of elevation data, featuring a 30 m spatial resolution in GeoTIFF format (Tachikawa et al. 2011; NASA/METI/AIST/Japan Space systems, 2019). Slope and aspect data were derived from the DEM using GIS tools. A total of 19 bioclimatic variables and three remotely sensed variables were used in the MaxEnt analysis (Table 1) (Hijmans et al. 2005; Fick and Hijmans, 2017; WorldClim, 2018). The DEM data were processed to correct anomalies, remove clouds, and generate uniform elevation values across all tiles, while reductions in water area anomalies are based on updated global water body data (Anonymous, 2023). Bioclimatic variables for current and projected 2070 conditions were obtained from WorldClim, compiled from observed monthly climate data for 1950–2000, while future projections were based on downscaled outputs of the HadGEM2-ES model under the RCP 6.0 scenario (Jones et al. 2011; van Vuuren et al. 2011; Fick and Hijmans, 2017). These variables, including temperature and precipitation measures, provide an ecologically relevant framework for assessing potential climate-driven distribution patterns of species (Hijmans et al. 2005; Fick and Hijmans, 2017).

The selected environmental variables reflect key ecological drivers known to influence free-living amoebae like *Acanthamoeba*. Topographic features such as elevation, slope, and aspect modulate microclimatic conditions and moisture retention, shaping suitable microhabitats (Fan et al. 2024). Climatic variables especially precipitation seasonality and temperature extremes are critical because *Acanthamoeba* exhibits encystment and dormancy responses under unfavorable moisture or thermal stress (Samba-Louaka, 2023). Moreover, free-living amoebae are known to persist across broad environmental gradients but are filtered by hydrological and substrate constraints (Salazar-Ardiles et al. 2022). By combining both terrain and bioclimatic predictors, the model aims to capture the environmental heterogeneity influencing *Acanthamoeba* distribution patterns.

Table 1. Environmental data (bioclimatic factors between 2011 and 2022 that could affect the presence of *Acanthamoeba*)

Name of data	Description
DEM	Digital elevation model (m)
Slope	Slope in degrees obtained from altitude (%)
Aspect	Aspect in degrees obtained from altitude (direction)
BIO1	Annual mean temperature (°C)
BIO2	Mean diurnal range (mean of monthly (max temp—min temp)) (°C)
BIO3	Isothermality (BIO2/BIO7) (× 100)
BIO4	Temperature seasonality (standard deviation × 100)
BIO5	Max temperature of warmest month (°C)
BIO6	Min temperature of coldest month (°C)
BIO7	Temperature annual range (BIO5-BIO6) (°C)
BIO8	Mean temperature of wettest quarter (°C)
BIO9	Mean temperature of driest quarter (°C)
BIO10	Mean temperature of warmest quarter (°C)
BIO11	Mean temperature of coldest quarter (°C)
BIO12	Annual precipitation (mm)
BIO13	Precipitation of wettest month (mm)
BIO14	Precipitation of driest month (mm)
BIO15	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter (mm)
BIO17	Precipitation of driest quarter (mm)
BIO18	Precipitation of warmest quarter (mm)
BIO19	Precipitation of coldest quarter (mm)

Species distribution modeling was performed using MaxEnt v3.4.1 (Phillips et al. 2017), which predicts where a species can live based on presence only data and environmental variables. We created a model using current species records and climate data to describe the species' habitat preferences. This model was then projected onto future climate conditions for 2070. For future climate data, we used the MIROC5 global climate model (Watanabe et al. 2010), developed by the University of Tokyo. The climate scenario used was RCP 4.5, which assumes moderate greenhouse gas emissions stabilizing by 2100 (IPCC, 2014). This approach is commonly used to study how species distributions might change under moderate climate change (Elith and Leathwick, 2009; Araújo and Peterson, 2012).

Ecological Niche Modeling of *Acanthamoeba* Using MaxEnt

The maximum entropy models were run using MaxEnt v3.4.1, which is publicly available at https://biodiversityinformatics.amnh.org/open_source/maxent/. No variable reduction was performed prior to modeling. All environmental parameters were included in the analysis, and the factors identified as most influential were determined based on the model outputs using the Jackknife test (Phillips et al. 2017; Almasieh et al. 2018).

In line with the study of Sofizadeh et al. (2017), the modeling framework utilized 74.46% of the presence points of *Acanthamoeba* species across 40 distinct locations, while the remaining 25% of the data was reserved for model evaluation.

The software was executed with the default settings, which included 10,000 background points, 0.00001 convergent thresholds, 15 replicates, and 5,000 iterations. The output was in the form of a logistic function that displayed a continuous probability of presence between 0 and 1. A jackknife approach was employed to determine the individual contribution of each variable in the modeling process. Model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC), which quantifies the model's ability to discriminate between presence and background points. This method is widely used in species distribution modeling (Phillips et al. 2006). However, it should be noted that AUC values in presence only models such as MaxEnt are calculated using background data rather than true absences, which may limit their interpretation. Despite this limitation, AUC remains a useful metric for relative model comparison in ecological niche modeling (Elith et al. 2006; Raes and ter Steege, 2007). The cartographic representations show the predicted likelihood of *Acanthamoeba* species occurrence for present day and 2070 scenarios. These maps are based on continuous probability values rather than binary conversions. Threshold-based binarization may cause information loss and reduce interpretability of spatial variation in model outputs.

Results

Isolation of *Acanthamoeba* from Both Soil and Water Samples

Soil and water samples from Afyon and Kütahya, Türkiye, underwent simple plating assays to ascertain the presence of *Acanthamoeba*. *Acanthamoeba* were identified in environmental samples (80% in water, 60% in soil) tested, based on culturing methodology (Fig. 2).

The culturing methodology revealed that 6 water specimens (60%) in Afyon and 10 water specimens (100%) in Kütahya tested positive for *Acanthamoeba* isolates. Furthermore, 7 soil specimens (70%) in Afyon and 5 soil specimens (50%) in Kütahya were contaminated with *Acanthamoeba* (Table 2).

18S rRNA-DNA sequencing and phylogenetic analysis

The genetic diversity among *Acanthamoeba* isolates was evident through genotyping analysis, revealing three distinct clusters: T3, T4, and T15. Notably, T4 isolates from Afyon clustered with strains from Kütahya. T3 from Kütahya was grouped in different clusters (Fig. 3).

MaxEnt Models

Risk maps for current and future projections were generated using the locations of culture-positive soil and water samples, without taking into account the specific genotypes (T3, T4, and T15) of *Acanthamoeba*. Figure 4 displays the ENM for both the present and anticipated (2070) timeframes, utilizing MaxEnt software and providing specific information regarding the distribution of *Acanthamoeba*.

The model indicates the current likelihood of *Acanthamoeba* isolates' occurrence in the southwestern region of Kütahya and the central area of Afyon. However, the

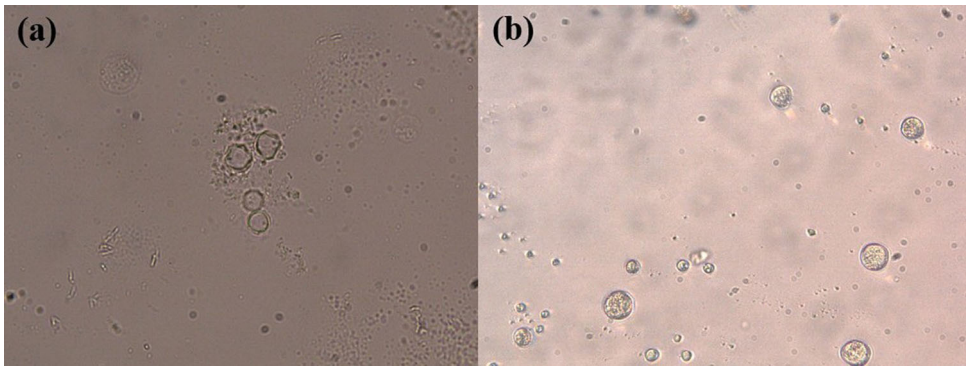


Figure 2. *Acanthamoeba* cysts that were observed under an inverted microscope (40X) on non-nutrient agar plates. The samples analyzed were water and soil, as indicated in panels (**a**) and (**b**), respectively.

Table 2. Results of detection of *Acanthamoeba*'s DNA by culture methods and 18S rRNA-DNA sequencing from water and soil samples

Province	Water	Soil	Water, (%)			Soil, (%)		
	Positive, (%)	Positive, (%)	T3	T4	T15	T3	T4	T15
Afyon	(6/10), 60	(7/10), 70	0	(6/6), 100	0	0	(7/7), 100	0
Kütahya	(10/10), 100	(5/10), 50	(1/10), 10	(8/10), 80	(1/10), 10	(1/5), 2 0	(5/4), 80	0

anticipated *Acanthamoeba* presence appears to be more widespread in both districts.

The current AUC values for both the training and testing datasets were computed to be 0.963 and 0.991, respectively, indicating the model's reliability. Using the model trained on present day data, future projections under the 2070 climate scenario were made. According to the findings presented in Fig. 5, the AUC of the training points was 0.963, while the predicted test points for 2070 achieved an AUC of 0.977.

The results of the Jackknife test revealed that, in the current model, the most influential environmental variables were BIO4 (temperature seasonality: standard deviation × 100, range 200–1000), BIO13 (precipitation of the wettest month: 50–400 mm), and BIO15 (precipitation seasonality: coefficient of variation, 0–100%). These variables highlight *Acanthamoeba*'s ecological sensitivity to thermal fluctuations and moisture availability, which are crucial for the survival and proliferation of free-living amoebae in natural habitats. Temperature seasonality (BIO4) indicates the degree of annual variation in temperature, while BIO13 reflects the abundance of water during peak rainfall periods, supporting the persistence of cysts and trophozoites. BIO15, representing variability in monthly precipitation, captures the predictability of moisture across seasons, which may affect the long-term stability of microhabitats. In the 2070 climate projection, BIO3 (isothermality: ratio of day-to-night temperature variation to annual temperature range, values between 20 and 60) also emerged as a key factor alongside BIO4, BIO13, and BIO15. The relationships between environmental variables remained consistent across time periods; however, the predicted distribution of *Acanthamoeba* shifted in response to projected climatic changes.

As shown in Fig. 6, the training gain for both models was approximately 1.5, indicating a moderate level of overall model accuracy when all variables were included. Figure 7 illustrates the seven environmental factors that elicit a response from *Acanthamoeba* in both present and projected future conditions, specifically in the year 2070. Currently, there exists a curvilinear correlation between BIO4 and the incidence of *Acanthamoeba*. Conversely, an inverse relationship was observed between BIO13, BIO15, and the presence of *Acanthamoeba*. The presence of *Acanthamoeba* in the future is associated with a curvilinear correlation with BIO3 and BIO4, while BIO13 and BIO15 continue to exhibit an inverse relationship with the likelihood of isolates.

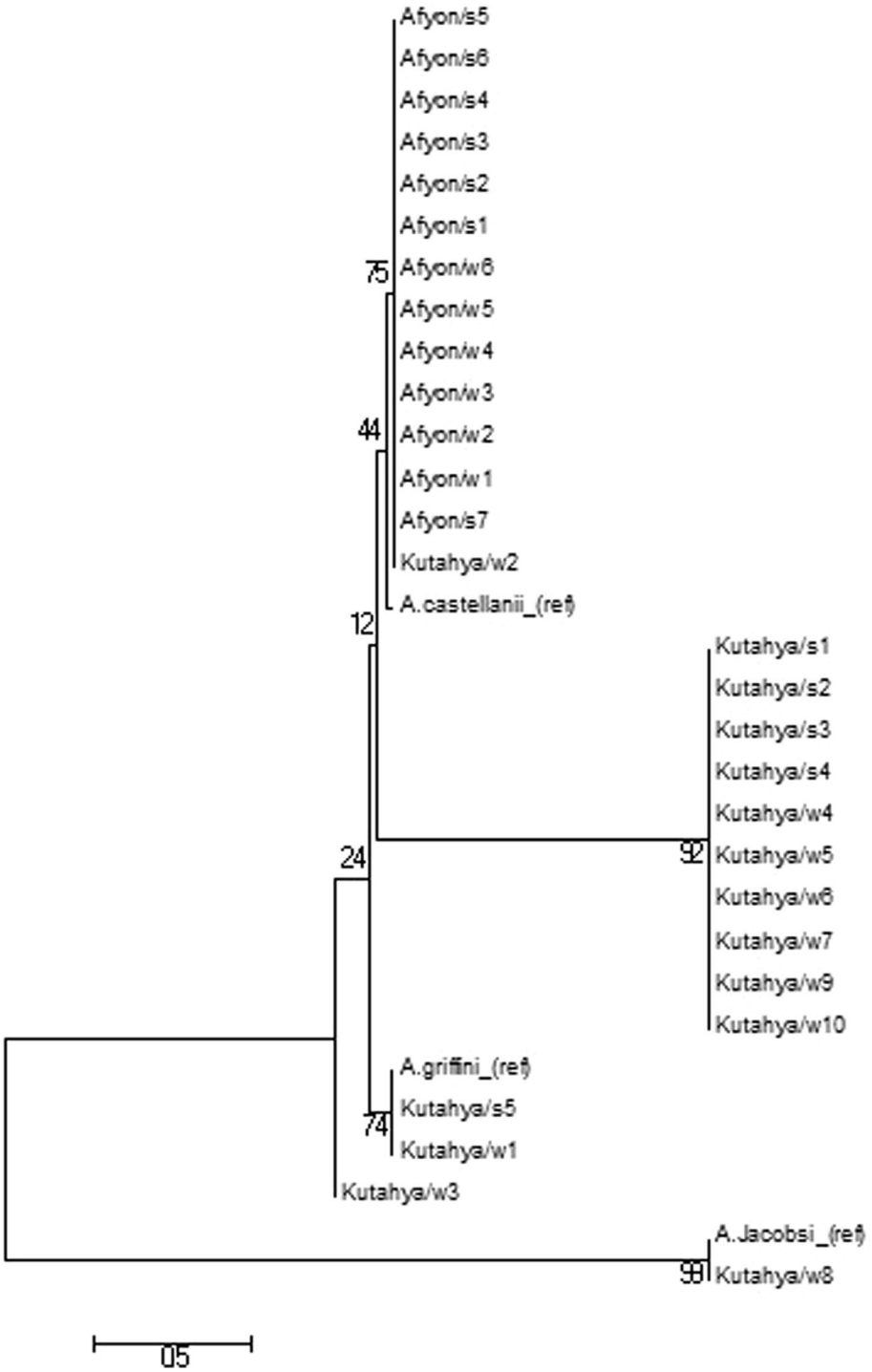


Figure 3. A neighbor-joining tree was constructed using MEGA11 software to analyze the 18S rRNA-DNA sequences of isolates from Afyon and Kütahya. Bootstrap values were generated from 1000 replications (S: soil samples; W: water samples).

Discussion

This study is the first to use MaxEnt modeling to investigate the potential geographic distribution of *Acanthamoeba* strains T3, T4, and T15 in Türkiye. It provides new insights into their environmentally suitable habitats under current and future climatic scenarios. The study also demonstrates the applicability of ENM for epidemiological surveys of pathogenic *Acanthamoeba*.

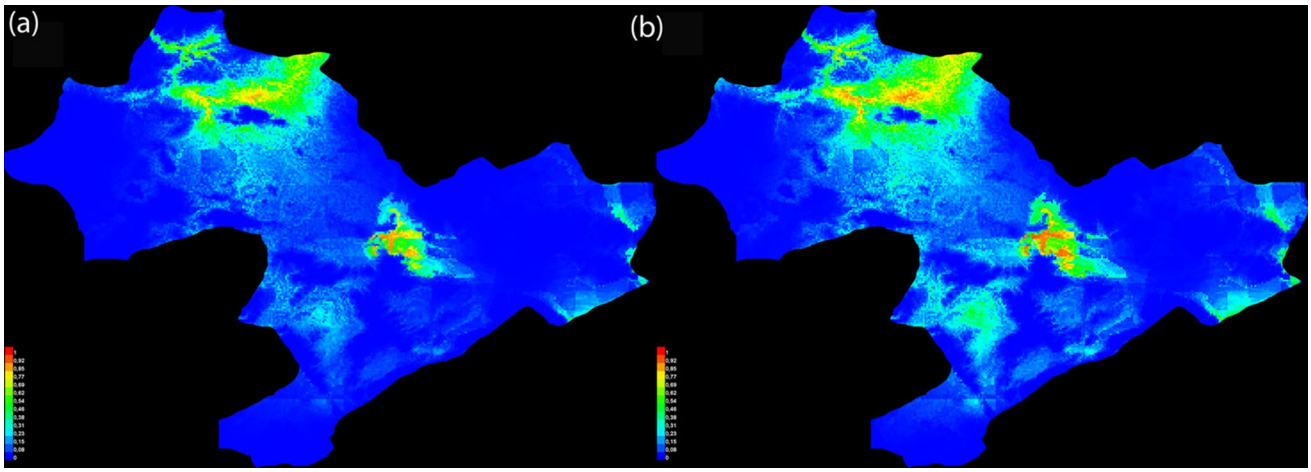


Figure 4. Spatial distribution of *Acanthamoeba* in Afyon and Kütahya provinces during the current period (**a**) and projected future period of 2070 under the RCP 4.5 climate scenario (**b**), predicted using Ecological Niche Modeling (ENM). The color gradient represents habitat suitability, with warmer colors (red) indicating higher suitability and cooler colors (blue) indicating lower suitability. Geographic boundaries and scales are shown for reference.

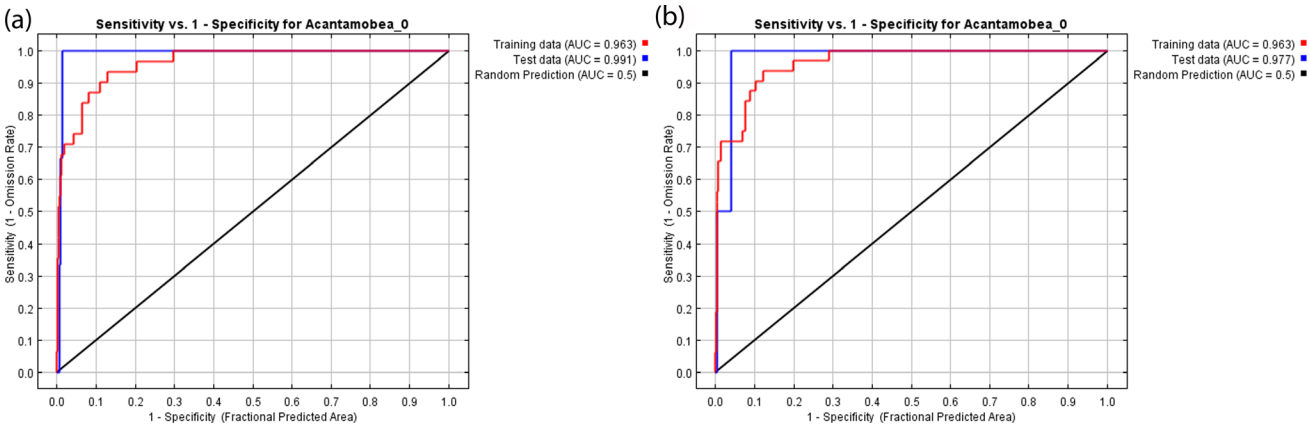


Figure 5. Area under the curve (AUC) of the Maximum Entropy (MaxEnt) model for *Acanthamoeba* in the regions of Afyon and Kütahya, Türkiye. Panel (**a**) represents the AUC for the present time, while panel (**b**) represents the AUC for the projected future time of 2070.

Following the predictive MaxEnt modeling, field sampling was conducted to verify the presence of *Acanthamoeba* strains in the study regions. In our study, strains T3, T4, and T15 were detected in Afyon and Kütahya (Table 2). These findings are consistent with previous reports from nearby regions, such as Ankara, where T2, T3, T4, and T7 genotypes were identified in 18 out of 28 soil samples collected from plant pots, as well as in two water samples, based on the 18S rRNA gene (Kılıç et al. 2004). Similarly, in Sivas, which shares climatic characteristics with our study areas, the T4 strain was also detected (Coşkun et al. 2013). Collectively, these observations corroborate the predictive distribution patterns generated by MaxEnt and highlight the environmental suitability of these regions for *Acanthamoeba* colonization.

The niche modeling results indicated that isothermality (BIO3), temperature seasonality (BIO4), precipitation of the wettest month (BIO13), and precipitation seasonality (BIO15) were the most influential predictors of *Acanthamoeba* distribution. These findings corroborate previous studies on thermotolerance and osmotolerance (Kahraman and Akın Polat, 2024), suggesting that the detected T3, T4, and T15 strains can withstand variable temperature and moisture conditions, thereby supporting their ecological adaptability and potential persistence in diverse habitats.

The positive detection rates of *Acanthamoeba* in soil and water samples from Afyon and Kütahya further support the ecological adaptability of these species. In Afyon, 60% of water samples and 70% of soil samples tested positive, with T4 being the predominant genotype in both

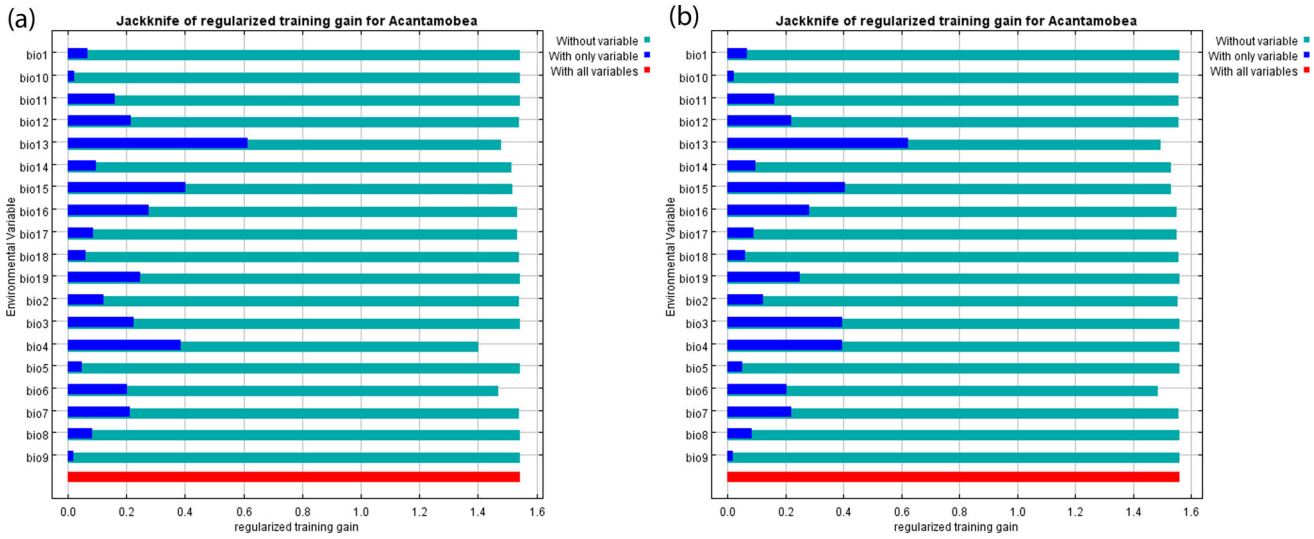


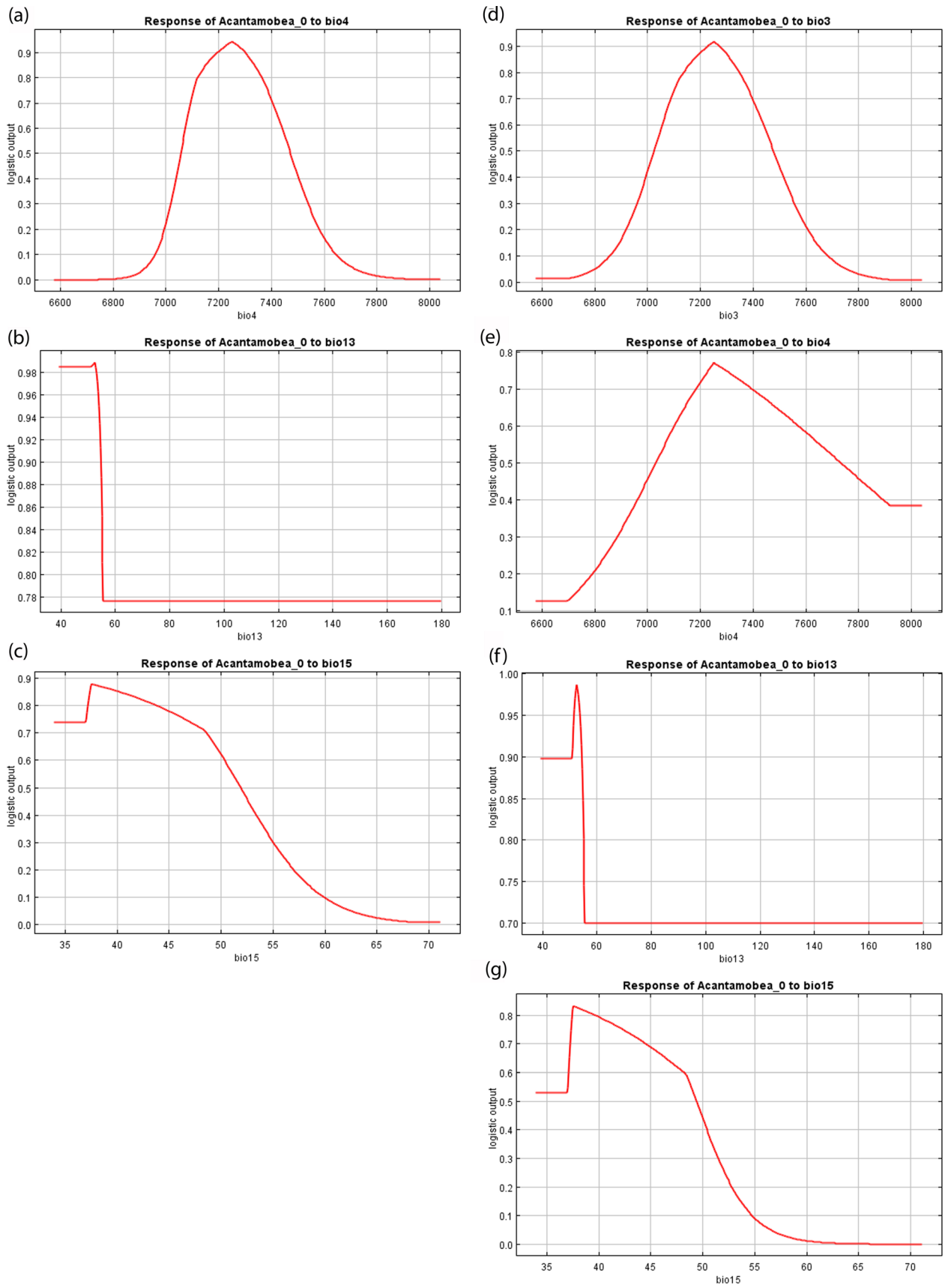
Figure 6. Jackknife test conducted on the MaxEnt model for *Acanthamoeba*, with panel (**a**) representing the present and panel (**b**) representing the projected future in 2070.

water (100%) and soil (100%). In Kütahya, all water samples (100%) and 50% of soil samples were positive, with T3, T4, and T15 also detected at varying frequencies (Table 2). These local findings align with the global distribution of *Acanthamoeba*. The species has been reported in many environmental niches, including soil, dust, air, natural and treated water, seawater, swimming pools, sewage, sediments, air-conditioning units, domestic tap water, drinking water treatment plants, bottled water, dental treatment units, hospitals, dialysis units, eyewash stations, and contact lenses and lens cases (Marciano-Cabral and Cabral, 2003). The worldwide prevalence of *Acanthamoeba* exhibits significant variability due to factors including water temperature, soil composition, climatic conditions, and diagnostic techniques (Kialashaki et al. 2018). These observations underscore the importance of monitoring both natural and anthropogenic habitats to understand the risk of human exposure and potential public health implications.

The outcomes of our research have the potential to provide epidemiological support for numerous investigations into *Acanthamoeba*, with primary data focusing on their distribution in the central Anatolian region. Several computer software programs, including ArcGIS, GARP, and MaxEnt, were utilized for the geographical analysis of acquired data and conducting fieldwork for diverse objectives. In the MaxEnt program, risk maps for *Acanthamoeba* were generated for both the present day and 2070, incorporating all environmental parameters included in the analysis. The factors identified as most influential in *Acanthamoeba* distribution were determined based on the model outputs using the Jackknife test, and were used to make risk predictions (Phillips et al. 2006).

The current model was entirely constructed based on environmental parameters supporting the presence of *Acanthamoeba* in areas where fieldwork was conducted, without differentiation between strains. Additionally, the time elapsed from when we collected the examples to the present day is considered too short for significant changes in environmental conditions, thus obviating the need for collecting new examples for the present (El-Sayed and Kamel, 2020). If the authors want to use this Ecological Niche Model to predict *Acanthamoeba* infection risks in a specific population, they must culture their specimens at elevated temperatures (35–37 °C). The culture temperature used in this study was 27 °C, in which both pathogenic and non-pathogenic *Acanthamoeba* can grow well. Hence, the present findings could only claim that their model was able to predict the occurrence of *Acanthamoeba* in the environment, but not directed to the pathogenic strains/risk of infections (Fig. 7).

It has been ascertained in both the United Kingdom and the United States that there is an escalation in the incidence of new cases attributed to *Acanthamoeba* during the summer and early autumn seasons. Additionally, it was observed that there were biannual peak periods during the spring and autumn seasons. Conversely, Ibrahim et al. reported that the coldest period of the year was associated with earlier symptom onset (Ibrahim et al. 2007). Our findings show a curvilinear association between *Acan-*


(a)
Response of Acantamobea_0 to bio4
logistic output
bio4
(d)
Response of Acantamobea_0 to bio3
logistic output
bio3
(b)
Response of Acantamobea_0 to bio13
logistic output
bio13
(e)
Response of Acantamobea_0 to bio4
logistic output
bio4
(c)
Response of Acantamobea_0 to bio15
logistic output
bio15
(f)
Response of Acantamobea_0 to bio13
logistic output
bio13
(g)
Response of Acantamobea_0 to bio15
logistic output
bio15

◀**Figure 7.** Response curves from the MaxEnt model showing the effect of the environmental factors with the greatest impact on *Acanthamoeba*. **a** The seasonality of temperature; **b** The amount of precipitation during the wettest month; **c** The seasonality of precipitation in the present era; **d** The degree of isothermality; **e** The seasonality of temperature; **f** The amount of precipitation during the wettest period; and **g** The seasonality of precipitation in the year 2070.

thamoeba presence and temperature. The occurrence of *Acanthamoeba spp*. exhibits a negative correlation with precipitation (Fig. 7). It is highly probable that the incidence of *Acanthamoeba* species cases will rise in the regions under our study. These regions are important for spa tourism. The increase is expected during the summer season and in 2070 due to anticipated temperature surges from global climate change.

Figure 7 shows seven environmental factors affecting *Acanthamoeba* under current and 2070 conditions. Currently, BIO4 shows a curvilinear correlation with *Acanthamoeba* presence, while BIO13 and BIO15 exhibit inverse relationships. In 2070 projections, BIO3 and BIO4 remain positively associated, whereas BIO13 and BIO15 continue to show inverse relationships, highlighting key determinants of *Acanthamoeba* distribution. It should be emphasized that the relationships among environmental variables themselves do not change in the 2070 projections. Instead, the differences observed reflect ecological responses of *Acanthamoeba* to altered temperature and precipitation regimes, rather than new or modified interactions among the predictor variables. This highlights that projected climatic changes primarily influence the organism's potential distribution rather than the interdependence of environmental factors. While certain research studies assert that temperature in isolation cannot account for the incidence of *Acanthamoeba,* others contend that fluctuations in precipitation and temperature are significant factors in the dissemination of *Acanthamoeba.* The findings indicate that the occurrence of *Acanthamoeba* is influenced by the combination of seasonal precipitation and temperature (Bharathi et al. 2007; Magnet et al. 2013).

In our study, cysts were detected in soil and water samples collected from Afyon and Kütahya (Table 2). Similar detections have been reported in nearby regions, including Ankara, where 18 out of 28 soil samples from plant pots and two water samples were positive (Kilic et al. 2004), and in Sivas, which has climatic conditions comparable to our study areas (Coşkun et al. 2013).

Despite these findings, a significant limitation of this study is the lack of external validation using independent environmental occurrence datasets of *Acanthamoeba* from Türkiye or adjacent regions. Such validation is essential to assess the generalizability and robustness of species distribution models beyond the training data (Araújo and Guisan, 2012; Wenger and Olden, 2012). However, due to the scarcity of publicly available records in the region, it could not be performed. Similar challenges have often been reported in niche modeling studies of understudied microorganisms or rare species (Pearson et al. 2007). Nevertheless, internal validation metrics, such as high Area Under the Curve (AUC) values, indicate acceptable model performance within the available dataset (Phillips et al. 2006). We acknowledge that AUC has limitations in presence only models such as MaxEnt, as it relies on background rather than true absence data (Lobo et al. 2008). Comparison with null models would provide a more robust assessment of model performance and is recommended for future studies (Raes and ter Steege, 2007). We emphasize the need for future research to expand occurrence databases and perform external validations to enhance model reliability and predictive accuracy.

A relatively small sample size, such as the 40 sampling points used in this study, can limit the robustness and generalizability of ecological niche models (ENMs) (Wisz et al. 2008). Although MaxEnt has been demonstrated to perform well with limited presence only data (Phillips et al. 2006), small datasets may still increase the risk of overfitting, particularly when a large number of environmental variables are included (Peterson et al. 2011). Although a random sampling design was used to minimize sampling bias, we acknowledge that the limited number of occurrence points may not fully eliminate potential spatial sampling bias. High AUC values, while indicative of good model fit, do not exclude overfitting or sampling bias (Lobo et al. 2008). Future studies should strive to increase sampling effort and incorporate independent data for external validation to improve model reliability (Elith et al. 2006).

Ecological niche modeling (ENM) approaches, such as MaxEnt and Random Forest, provide actionable insights for public health planning by identifying areas with elevated disease risk based on environmental, climatic, and socio-economic predictors. For example, the SFTS risk mapping in China highlighted regions where surveillance

and targeted vector control should be prioritized (Liu et al. 2015). Similarly, environmental suitability modeling for scrub typhus in Nepal revealed high-risk lowland and river valley areas, informing early warning systems and allocation of diagnostic resources (Acharya et al. 2019). In Bolivia, the integration of socio-economic variables into Chagas disease models allowed the identification of communities at heightened risk, supporting the planning of vector control campaigns and community education programs (Mischler et al. 2012). Moreover, modeling soil-transmitted helminth distributions in Thailand identified high-risk provinces, guiding mass drug administration and sanitation interventions (Chaiyos et al. 2018). Collectively, these studies illustrate that ENM outputs can be directly integrated into evidence-based public health decision-making, enabling the prioritization of surveillance, targeted interventions, and resource allocation in areas predicted to be at elevated risk. Therefore, we consider that the present study's predictions regarding the environmental suitability of *Acanthamoeba* can inform the identification of high-risk zones and support the planning of preventive public health strategies. Public health professionals can use the predicted environmental suitability maps to identify areas with elevated *Acanthamoeba* exposure risk. These high-risk zones can guide targeted surveillance, prioritization of diagnostic testing, and public awareness campaigns. By integrating these predictions into local health planning, authorities can allocate resources efficiently, implement preventive strategies in vulnerable communities, and monitor environmental changes that may increase infection risk.

Conclusion

Ecological niche models and mapping technologies play a crucial role in evaluating and mapping the status of various diseases. In this study, the risk maps generated for *Acanthamoeba* using MaxEnt may prove to be valuable tools for healthcare professionals and researchers in strategizing, administering, and regulating monitoring and control measures in regions where *Acanthamoeba* is prevalent. One potential application is the implementation of surveillance programs aimed at monitoring the spread of *Acanthamoeba*, gaining a deeper understanding of its environmental distribution, and assessing associated epidemiological patterns and risk levels. Moreover, the risk maps generated in this study could also help anticipate potential economic impacts related to infection control and prevention.

The bioclimatic features identified in our research on two Turkish municipalities, which account for the presence of species, will help indicate areas with higher risk of diseases associated with *Acanthamoeba*, rather than directly mapping disease prevalence.

Author Contributions

HK and GE designed the research, carried out most experiments, performed analyses, and wrote the first draft of the article. HK and OA designed, carried out, and analyzed data from the prey choice experiment. GE collected specimens. OA collected bioclimatic data. GE, HK and OA designed the research and advised on data analysis. All authors contributed to writing and revising the manuscript.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Approval This study did not involve human participants or animal subjects. Therefore, ethical approval was not required.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

References

Acharya BK, Cao C, Xu M, Khanal L, Naeem S, Pandit SR, Adhikari BR (2019) Environmental determinants of scrub typhus

occurrence and vector distribution in Nepal. *Ecological Informatics* 52:151–161. https://doi.org/10.1016/j.ecoinf.2019.05.004

Akbulut G (2010) The thermal spring tourism in Turkey and problems. *Gaziantep Univ J Soc Sci* 9:35–54

Almasieh K, Zoratipour A, Negaresh K, Delfan-Hasanzadeh K (2018) Habitat quality modeling and effect of climate change on the distribution of Centaurea pabotii in Iran. *Span J Agric Res* 16(3):e0304. https://doi.org/10.5424/sjar/2018163-13098

Alves DSMM, Moraes AS, Nitz N, Oliveira MGC, Hecht MM, Gonçalves RG, Ferreira LR, Silva AC, Santos SM, Costa RT (2012) Occurrence and characterization of *Acanthamoeba* similar to genotypes T4, T5, and T2/T6 isolated from environmental sources in Brasília, Federal District, Brazil. *Exp Parasitol* 131:239–244. https://doi.org/10.1016/j.exppara.2012.04.011

Anonymous. https://www.earthdata.nasa.gov/news/new-aster-gdem (Accessed February 2023).

Araujo MB, Peterson AT (2012) Uses and misuses of bioclimatic envelope modeling. *Ecol* 93(7):1527–1539. https://doi.org/10.1890/11-1930.1

Aykur M, Dağcı H (2021) Evaluation of molecular characterization and phylogeny for quantification of *Acanthamoeba* and *Naegleria fowleri* in various water sources, Turkey. *PLOS ONE* 16(8):e0256659. https://doi.org/10.1371/journal.pone.0256659

Bacon AS, Frazer DG, Dart JK, Matheson M, Ficker LA, Wright P (1993) A review of 72 consecutive cases of *Acanthamoeba keratitis* 1984–1992. *Eye* 7:719–725

Basher MHA, Ithoi I, Mahmud R, Abdulsalam AM, Foead AI, Dawaki S, Ali R, Hassan R, Abdullah N, Omar N, Rahman S (2018) Occurrence of *Acanthamoeba* genotypes in Central West Malaysian environments. *Acta Trop* 178:219–228. https://doi.org/10.1016/j.actatropica.2017.11.015

Bharathi JM, Srinivasan M, Ramakrishnan R, Meenakshi R, Padmavathy S, Lalitha P, Madhavan HN (2007) A study of the spectrum of *Acanthamoeba keratitis*: a three-year study at a tertiary eye care referral center in South India. *Indian J Ophthalmol* 55:37–42. https://doi.org/10.4103/0301-4738.29493

Booton GC, Visvesvara GS, Byers TJ, Kelly DJ, Fuerst PA (2005) Identification and distribution of *Acanthamoeba* species genotypes associated with nonkeratitis infections. *J Clin Microbiol* 43:1689–1693. https://doi.org/10.1128/jcm.43.4.1689-1693.2005

Bouheraoua N, Labbé A, Chaumeil C, Liang Q, Laroche L, Le Hoang P, Dos Santos L, Proust B (2014) Acanthamoeba keratitis. *J Fr Ophtalmol* 37:640–652. https://doi.org/10.1016/j.jfo.2014.05.004

Carnt N, Robaei D, Minassian DC, Dart JKG (2018) *Acanthamoeba keratitis* in 194 patients: risk factors for bad outcomes and severe inflammatory complications. *Br J Ophthalmol* 102:1431–1435. https://doi.org/10.1136/bjophthalmol-2017-310806

Chaiyos J, Rongsriyam Y, Limsomboon J, Thongwat D, Apiwathnasorn C, Choochote W, Samung Y (2018) Modeling potential distribution of soil-transmitted helminths in Thailand using ecological niche modeling. *Acta Tropica* 182:1–8. https://doi.org/10.1016/j.actatropica.2018.02.020

Coşkun KA, Ozçelik S, Tutar L, Elaldı N, Tutar Y (2013) Isolation and identification of free-living amoebae from tap water in Sivas, Turkey. *Biomed Res Int* . https://doi.org/10.1155/2013/675145

Dart JK, Saw VP, Kilvington S (2009) *Acanthamoeba keratitis*: diagnosis and treatment update 2009. *Am J Ophthalmol* 148:487–499. https://doi.org/10.1016/j.ajo.2009.06.009

Dirim Erdoğan D, Aykur M (2022) The risk factors and clinical features of *Acanthamoeba* keratitis: first time detection of *Acanthamoeba* T5 genotype from keratitis patients in Turkey. *Acta Parasitol* 67:1384–1392. https://doi.org/10.1007/s11686-022-00596-4

Dos Santos DL, Kwitko S, Marinho DR, de Araújo BS, Locatelli CI, Rott MB (2018) *Acanthamoeba keratitis* in Porto Alegre (southern Brazil): 28 cases and risk factors. *Parasitol Res* 117:747–750. https://doi.org/10.1007/s00436-017-5745-y

Elith J, Leathwick JR (2009) Species distribution models: Ecological explanation and prediction across space and time. *Annu Rev Ecol Evol Syst* 40:677–697. https://doi.org/10.1146/annurev.ecolsys.110308.120159

Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick J, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Overton JM, Peterson AT, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29:129–151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

El-Sayed K, Kamel M (2020) Climatic changes and their role in emergence and re-emergence of diseases. *Environ Sci Pollut Res* 27:22336–22352. https://doi.org/10.1007/s11356-020-08896-w

Esboei BR, Fakhar M, Saberi R, Barati M, Moslemi M, Hassannia H, Sharifi I, Ghaffari S, Khatami A, Ziaei H (2020) Genotyping and phylogenic study of *Acanthamoeba* isolates from human keratitis and swimming pool water samples in Iran. *Parasite Epidemiol Control* 11:e00164. https://doi.org/10.1016/j.parepi.2020.e00164

Fan S, Shen Y, Qian L (2024) Social life of free-living amoebae in aquatic environment comprehensive insights into interactions of free-living amoebae with neighboring microorganisms. *Front. Microbiol* 15:1234567. https://doi.org/10.3389/fmicb.2024.1234567

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int J Climatol* 37(12):4302–4315. https://doi.org/10.1002/joc.5086

Glass GE (2001) Geographic information systems. In: Nelson KE, Williams CM, editors. Infectious disease epidemiology: Theory and practice. Gaithersburg, MD: Aspen Publishers 2001; p. 231–53.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25(15):1965–1978. https://doi.org/10.1002/joc.1276

Ibrahim YW, Boase DL, Cree IA (2007) Factors affecting the epidemiology of *Acanthamoeba keratitis*. *Ophthalmic Epidemiol* 14:53–60. https://doi.org/10.1080/09286580600920281

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: IPCC.

Jones CD, Hughes JK, Bellouin N, Hardiman SC, Jones GS, Knight J, Liddicoat S, O'Connor FM, Andres RJ, Bell C, Boo KO, Bozzo A, Butchart N, Cadule P, Corbin KD, DoutriauxBoucher M, Friedlingstein P, Gornall J, Gray L, Halloran PR, Hurtt G, Ingram WJ, Lamarque JF, Law RM, Meinshausen M, Osprey S, Palin EJ, Parsons Chini L, Raddatz T, Sanderson MG, Sellar AA, Schurer A, Valdes P, Wood N, Woodward S, Yoshioka M, Zerroukat M (2011) The HadGEM2-ES implementation of CMIP5 centennial simulations. *Geoscientific Model Development* 4(3):543–570. https://doi.org/10.5194/gmd-4-543-2011

Kahraman M, Akın Polat Z (2024) Are Thermotolerant and Osmotolerant Characteristics of *Acanthamoeba* Species an

Indicator of Pathogenicity. *Turk Parazitol Derg* 48(1):15–20. https://doi.org/10.4274/tpd.galenos.2024.92408

Kalra SK, Sharma P, Shyam K, Tejan N, Ghoshal U (2020) *Acanthamoeba* and its pathogenic role in granulomatous amebic encephalitis. *Exp Parasitol* 208:107788. https://doi.org/10.1016/j.exppara.2019.107788

Kialashaki E, Daryani A, Sharif M, Gholami S, Dodangeh S, Mohebali M, Zia-Ali N, Rezaeian M (2018) *Acanthamoeba spp.* from water and soil sources in Iran: a systematic review and meta-analysis. *Ann Parasitol* 64:285–297. https://doi.org/10.17420/ap6404.163

Kilic A, Tanyuksel M, Sissons J, Jayasekera S, Khan NA (2004) Isolation of *Acanthamoeba* isolates belonging to T2, T3, T4 and T7 genotypes from environmental samples in Ankara, Turkey. *Acta Parasitol* 49:246–252

Liu K, Zhou H, Sun RX, Yao HW, Li Y, Wang LP, Mu D, Zhang H, Li ZJ, Wang Y, Luo L, Cui N, Yang WZ, Gaowa HS, Cao WC, Yu HJ (2015) A national assessment of the epidemiology of severe fever with thrombocytopenia syndrome. *China. Scientific Reports* 5:9679. https://doi.org/10.1038/srep09679

Lobo JM, Jiménez-Valverde A, Real R (2008) AUC: a misleading measure of the performance of predictive distribution models. *Glob Ecol Biogeogr* 17(2):145–151. https://doi.org/10.1111/j.1466-8238.2007.00358.x

Lorenzo-Morales J, Ortega-Rivas A, Martínez E, Khoubbane M, Artigas P, Valladares B (2006) *Acanthamoeba* isolates belonging to T1, T2, T3, T4 and T7 genotypes from environmental freshwater samples in the Nile Delta region. *Egypt. Acta Trop* 100:63–69. https://doi.org/10.1016/j.actatropica.2006.09.008

Magnet A, Fenoy S, Galván AL, Izquierdo F, Rueda C, Vadillo CF, Del Río L, Mayorga P, Arias C (2013) A year long study of the presence of free living amoeba in Spain. *Water Res* 47:6966–6972. https://doi.org/10.1016/j.watres.2013.09.065

Marciano-Cabral F, Cabral G (2003) *Acanthamoeba spp.* as agents of disease in humans. *Clin Microbiol Rev* 16:273–307. https://doi.org/10.1128/cmr.16.2.273-307.2003

Meighani M, Eslamirad Z, Hajihossein R, Ahmadi A, Saki S (2018) Isolation and Genotyping of *Acanthamoeba* from Soil Samples in Markazi Province. *Iran. Open Access Maced J Med Sci* 6:2290–2294. https://doi.org/10.3889/oamjms.2018.454

Mischler P, Kearney M, Picollo MI, Borrás R, Gürtler RE (2012) Environmental and socio-economic determinants of Chagas disease vectors in the Gran Chaco region of Bolivia. *PLoS Neglected Tropical Diseases* 6(2):e1510. https://doi.org/10.1371/journal.pntd.0001510

NASA/METI/AIST/Japan Spacesystems (2019) ASTER Global Digital Elevation Model Version 3 (GDEM v3) . Land Processes Distributed Active Archive Center. https://lpdaac.usgs.gov/products/astgtmv003/.

Niederkorn JY (2021) The biology of *Acanthamoeba keratitis*. *Exp Eye Res* 202:108365. https://doi.org/10.1016/j.exer.2020.108365

Pana A, Vijayan V, Anilkumar AC (2023) *Amebic Meningoencephalitis*, Treasure Island: StatPearls Publishing

Pearson RG, Raxworthy CJ, Nakamura M, Peterson AT (2007) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *J Biogeogr* 34(1):102–117. https://doi.org/10.1111/j.1365-2699.2006.01594.x

Peterson AT, Papeş M, Eaton M (2011) Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. *Ecography* 34(4):411–422. https://doi.org/10.1111/j.1600-0587.2010.06302.x

Phillips SJ, Anderson RP, Schapired RE (2006) Maximum entropy modeling of species geographic distributions. *Ecol Modell* 190:231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Phillips SJ, Anderson RP, Dudík M, Schapire RE, Blair ME (2017) Opening the black box: An overview of MaxEnt software for modeling species distributions. *Ecography* 44(10):1501–1515. https://doi.org/10.1111/ecog.05688

Raes N, ter Steege H (2007) A null-model for significance testing of presence-only species distribution models. *Ecography* 30(5):727–736. https://doi.org/10.1111/j.2007.0906-7590.05041.x

Salazar-Ardiles C, Asserella-Rebollo L, Andrade DC (2022) Free-Living Amoebas in Extreme Environments: The True Survival in Our Planet. *Biomed Res Int* 2022:2359883. https://doi.org/10.1155/2022/2359883

Samba-Louaka A (2023) Encystment of Free-Living Amoebae, So Many Blind Spots to Cover. *Parasitologia* 3(1):1–15. https://doi.org/10.3390/parasitologia3010001

Schroeder JM, Booton GC, Hay J, Niszl IA, Seal DV (2001) Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of acanthamoebae from humans with keratitis and from sewage sludge. *J Clin Microbiol* 39:1903–1911. https://doi.org/10.1128/jcm.39.5.1903-1911.2001

Sofizadeh A, Rassi Y, Vatandoost H, Hanafi-Bojd AA, Mollalo A, Zare M, Oshaghi MA, Aghaie A, Haghdoost AA (2017) Predicting the distribution of Phlebotomus papatasi (Diptera: Psychodidae), the primary vector of zoonotic cutaneous leishmaniasis, in Golestan province of Iran using ecological niche modeling: comparison of MaxEnt and GARP models. *J Med Entomol* 54:312–320. https://doi.org/10.1093/jme/tjw178

Tachikawa T, Hato M, Kajiwara K, Iwasaki A (2011) ASTER Global DEM Version 2 – Summary of ASTER GDEM Production and Validation. *IEEE International Geoscience and Remote Sensing Symposium (IGARSS)* 2011:3657–3660. https://doi.org/10.1109/IGARSS.2011.6049807

van Vuuren DP, Edmonds J, Kainuma M, Riahi K, Thomson A, Hibbard K, Hurtt GC, Kram T, Krey V, Lamarque JF, Masui T, Meinshausen M, Nakicenovic N, Smith SJ, Rose SK (2011) The representative concentration pathways: an overview. *Climatic Change* 109(1–2):5–31. https://doi.org/10.1007/s10584-011-0148-z

Watanabe M, Suzuki T, O'Ishi R, Komuro Y, Watanabe S, Emori S, Takemura T (2010) Improved climate simulation by MIROC5: Mean states, variability, and climate sensitivity. *J Clim* 23(23):6312–6335. https://doi.org/10.1175/2010JCLI3679.1

Wenger SJ, Olden JD (2012) Assessing transferability of ecological models: an underappreciated aspect of statistical validation. *Methods Ecol Evol* 3(2):260–267. https://doi.org/10.1111/j.2041-210X.2011.00170.x

Wisz MS, Hijmans RJ, Li J, Peterson AT, Graham CH, Guisan A, NCEAS Predicting Species Distributions Working Group (2008) Effects of sample size on the performance of species distribution models. *Diversity Distrib* 14(5):763–773. https://doi.org/10.1111/j.1472-4642.2008.00482.x

WorldClim (2018) WorldClim-global climate data. http://www.worldclim.org/bioclim (Accessed 10 Febuary 2018).

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.