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TRABALHO DE CONCLUSÃO DE CURSO

Worldwide distribution of genotypes in *Acanthamoeba* keratitis: a systematic review

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Abstract

Acanthamoeba spp. are among the most prevalent protozoa distributed in the world, causing diseases as *Acanthamoeba* keratitis, which is a painful and severe sight threatening corneal disease that in some cases can cause even blindness. In recent years, the prevalence of *Acanthamoeba* keratitis has rapidly increased, making it increasingly recognized as important for human health. This systematic review proposes to analyze the frequency of genotypes of the genus *Acanthamoeba* in keratitis cases around the world, as well as to analyze the types of samples collected and the identification methods used. Most cases were found in Asia and Europe. Undoubtedly, the T4 genotype was the most prevalent worldwide, followed by T3, T15, T11 and T5. Besides that, T4 genotype was also related to a greater number of species of *Acanthamoeba*. Given the differences in pathogenicity, virulence,

susceptibility to treatment and clinical outcomes between genotypes, genotyping of all isolates from cases of *Acanthamoeba* keratitis is essential to have a better correlation between *in vitro* and *in vivo* efficacy, resulting in better drug therapies and successful treatment in cases of this important ocular infection.

Keywords: free living amoebas, *Acanthamoeba* spp., keratitis, genotype

Introduction

Free-living amoebae (FLA) of the genus *Acanthamoeba* are ubiquitously distributed in the nature (Siddiqui and Khan 2012). They can be isolated from practically all natural and artificial environments including soil, dust, water, air, medical equipment, lens fluids, air-conditioning and also in the nasopharyngeal mucosa of healthy individuals (Clarke and Niederkorn 2006; Nagyová et al. 2010a; Siddiqui and Khan 2012; Khezri et al. 2016; Tawfeek et al. 2016; Król-Turmińska and Olender 2017; Lass et al. 2017). *Acanthamoeba* spp. are among the most prevalent protozoa distributed in the world and are known as amphizoic organisms because they have the ability to exist both as free-living amoebae or as pathogenic and opportunistic parasites that often come into contact with humans, causing serious infections (Oddo 2006; Visvesvara et al. 2007; Lanocha et al. 2009).

Besides, they can cause infections by themselves, *Acanthamoeba* spp. are known to be "Trojan horses'', serving as hosts of a variety of pathogens microorganisms which may include viruses, protists and bacteria, may acting as a reservoir for maintaining and dispersing their endosymbionts in the environment (Greub and Raoult 2004; Berger et al. 2006; Siddiqui and Khan 2012).

As a parasite, *Acanthamoeba* can cause a clinical condition called granulomatous amebic encephalitis (GAE), which is an opportunistic, insidious and chronic infection of the

central nervous system characterized by relatively high mortality despite low incidence (Visvesvara et al. 2007; Visvesvara and Schuster 2008; Diaz 2010). These FLA can also cause several highly destructive disseminating infections concerning lungs, kidneys, liver, adrenal glands, heart, bones and skin, that can affect both immunocompromised and immunocompetent patients. However, the most common extracerebral infection caused by this amoeba is *Acanthamoeba* keratitis (AK) (Khan 2006; Ren and Wu 2010; Walochnik et al. 2015; Kot et al. 2018).

Acanthamoeba keratitis is a painful and severe sight threatening corneal disease that in some cases can cause even blindness. Unlike GAE, AK also occurs in immunocompetent individuals, as a result of poor hygiene in the care of contact lenses or after a corneal trauma (Visvesvara et al. 2007; Dart et al. 2009; Jercic et al. 2019). The clinical findings of AK include excruciating pain, considerable production of tears, photophobia, inflammation with redness, corneal abrasion and opacification, blurred vision, foreign body sensation, edema, stromal infiltration, epithelial loss, ring ulcers, cataract, glaucoma and even corneal perforation and vision loss if it's not treated aggressively and adequately (Khan 2006; Castrillón and Orozco 2013; Lorenzo-Morales et al. 2015). Meanwhile, the same symptoms can occur in bacterial, fungal and viral keratitis, making common the misdiagnosis, although the AK usually progresses slower than the others (Lorenzo-Morales et al. 2013). Besides that, cases of co-infection of *Acanthamoeba* spp. with fungi as *Fusarium* and *Candida* or bacteria as *Pseudomonas* have already been reported (Sharma et al. 2013; Nunes et al. 2016; Buchele et al. 2018).

In recent years, the prevalence of *Acanthamoeba* keratitis has rapidly increased, therefore it has become increasingly recognized as important in human health. The prevalence has increased due to availability of diagnostic methods that allow the differential diagnosis of other types of keratitis, as well as by the increase in the number of contact lens

(CL) users, which is the main risk factor for the disease (Khan 2006; Patel and Hammersmith 2008; Dart et al. 2009).

The number of contact lens users are growing every year worldwide and the number of AK cases has increased concomitantly with this number (Maycock and Jayaswal 2016). Currently, about 90% of patients diagnosed with AK are CL wearers, with reported rates between 1 to 33 cases per million. (Khan 2006; Visvesvara et al. 2007). Due to the fact that the amoeba gains access to the lens case through the air or tap water, AK infections related to CL are in most cases related to poor cleaning, overuse, swimming or sleeping with them. However, it is important to note that even patients who regularly disinfect the lens with multipurpose solution still can contract AK, since it has been shown that most commercially available cleaning solutions are ineffective against the protozoan (Kilvington et al. 2004; Hammersmith 2002; Shoff et al. 2008; Walochnik et al. 2015). One of the factors that can explain this situation is the biofilm formation after the contamination of contact lenses, which may enhance *Acanthamoeba* persistence on contact lens storage as well as providing nutrients for the amoeba, playing an important role in the pathogenesis of AK (Khan 2006). Other predisposing factors, even in users who don't wear contact lenses, include previous mechanical corneal trauma associated with the exposure of contaminated soil, water or vegetation (Jiang et al. 2006; Wesolowska et al. 2006; Lorenzo-Morales et al. 2015).

Diagnosis of AK is one of the most challenging corneal diseases to be diagnosed and is only often considered when there is a failure in the response to first line therapy for herpes simplex virus or bacterial/fungal keratitis. Besides that, diagnosis methods currently used are invasive because they require stromal biopsy or corneal scrapes, for example. Moreover, the sooner the disease is diagnosed, better are the chances of a successful prognosis (Dart et al. 2009; Page and Mathers 2013).

AK treatment is difficult and prolonged, becoming an extremely challenging problem due to the fact that there are no drugs specifically approved for this infection, so in general multiple antibacterial, antifungal and antiamoebic agents are used in combination to improve the results (Gokhale 2008; Wilhelmus et al. 2008; Juárez et al. 2018). It occurs because a lot of factors, including the wide range of virulence that the different genotypes of *Acanthamoeba* spp. show, make it almost impossible to establish a correlation between *in vivo* and *in vitro* drug activity efficacies. Beyond that, even after the clinical resolution, medications have to be used for a long time to prevent relapses, due to the fact that de cystic forms are extremely resistant (Kumar and Lloyd 2002; Astorga et al. 2011). In case of severe infection, corneal transplantation is the last therapeutic option when oral or topical treatments have failed (Kitzmann et al. 2009; Nguyen et al. 2010; Lorenzo-Morales et al. 2015). Among the measures that can be used to prevent the infection are educating lens wearers regarding the proper care of contact lenses and it's cases, using the appropriate disinfecting solutions, no overnight wear of CL and no showering or swimming with contact lenses, in order to avoid the contact with contaminated water (Visvesvara et al. 2007).

To avoid wrong treatments and early diagnosis, it is essential to know the pathogen. The life cycle of *Acanthamoeba* spp. consists in two stages, an actively feeding and reproduction trophozoite and a latent cyst stage, with minimal metabolic activity (Siddiqui and Khan 2012; Lorenzo-Morales et al. 2015). The trophozoites of *Acanthamoeba* spp. exhibit prominent vacuoles and typical acanthopodia, which are fine and spine-like structures on their surface. Their size is normally around 12–35 μm in diameter, but it can vary significantly between isolates, due to the many different genotypes and species (Khan 2006; Visvesvara et al. 2007; Costa et al. 2010). As a result of unfavourable environmental conditions as desiccation, changes in pH and temperature, increased osmolarity or hypo-osmolarity and food deprivation, the encystment occurs. Briefly, the trophozoite

becomes a cyst metabolically inactive, that has a double wall composed of an endocyst and ectocyst both containing cellulose (Marciano-Cabral and Cabral 2003; Munguía 2005; Siddiqui et al. 2012; Costa et al. 2010; Martín-Pérez et al. 2017). It allows the organism to survive extreme conditions, retaining its pathogenic properties for long periods of time in hostile environments, which justifies why AK treatment is so difficult, because *Acanthamoeba* encysts when the environment becomes unfavorable due to the medications. Both cysts and trophozoites can adhere to the surface, including soft or rigid CL and contact lens cases, allowing them to invade the eye tissues (Khan and Tareen 2003; Marciano-Cabral and Cabral 2003; Siddiqui and Khan 2012).

In an attempt to organize the increasing number of isolates belonging to the *Acanthamoeba* genus, Pussard and Pons (1977) initially classified the species based on morphological features of the cysts. Then, *Acanthamoeba* spp. were divided into three different morphological groups (I-III), according to their cyst shape of ectocyst and endocyst as well as the size. This methodology made it possible to differentiate more than 24 species of *Acanthamoeba* (Khan 2006; Visvesvara et al. 2007; Kłopocka et al. 2009; Fuerst et al. 2015; Derda et al. 2016). Most AK infections are caused by representatives of group II, but some isolates belonging to the group III have also been described as causative agents of the disease. Among the species of *Acanthamoeba* that cause AK, the most prevalent are *Acanthamoeba polyphaga* and *A. castellanii*, although *A. culbertsoni*, *A. rhysodes*, *A. griffini*, *A. quina* and *A. lugdunensis* have also been described as causing the infection (Clarke and Niederkorn 2006; Visvesvara 2010; Lorenzo-Morales et al. 2015). Nevertheless, the classification based on morphology criteria is currently considered ambiguous and unreliable because species morphology may change according to the culture conditions, resulting in variations in cyst morphology, which is a very important characteristic for species identification. Moreover, several studies have demonstrated inconsistencies in cyst

morphology of the same isolate, indicating that morphological identification should not be used alone for the identification of species, requiring the use of molecular techniques (Khan 2006; Castrillón and Orozco 2013). To avoid these problems, nowadays molecular classification methods have been generated, which are usually classified on the basis of the nuclear small subunit 18S ribosomal RNA full gene sequence (Rns), which allows the differentiation of *Acanthamoeba* spp. into 22 genotypes (T1-T22) and encompass all the *Acanthamoeba* isolates found so far (Corsaro et al. 2015; Fuerst and Booton 2015; Corsaro et al. 2017; Taher et al. 2018).

Several studies across the world suggest that the predominant genotype in both keratitis and non-keratitis samples, has been the T4 genotype. Meantime, T2, T3, T5, T6, T8, T9, T11, T13, and T15 genotype species have also been isolated from patients with AK. Therefore, most genotypes known to date have at least once been involved with the disease in humans, although in the best of our knowledge, there are no studies that have taken global cases into account jointly, but analyzed regionally (Khan et al. 2002; Maghsood et al. 2005; Iovieno et al. 2010; Booton et al. 2009; Risler et al. 2013; Walochnik et al. 2014).

Molecular techniques, especially sequencing of 18S rRNA genes are increasingly being used for *Acanthamoeba* genotyping. The sequences obtained are compared to *Acanthamoeba* reference strains sequences, through multiple alignments with all available 22 genotypes, with the model assumption of a <5% sequence dissimilarity within them (Visvesvara et al. 2007; Lorenzo-Morales et al. 2015). Besides that, PCR-based methods have been developed for the rapid and useful detection of *Acanthamoeba*, which have good sensitivity and specificity (Schroeder et al. 2001; Fraser et al. 2012). Genotypic classification can be achieved by exploiting the inter-strain variations in the 18S ribosomal RNA (rRNA) subunit (Rns) sequence. The complete Rns gene exceeds 2000 nucleotides, then a fragment within the Rns gene named as "*Acanthamoeba* specific amplimer (ASA.S1)'' with 423 to 551

bp is used for genotyping *Acanthamoeba* spp. (Schroeder et al. 2001; Booton et al. 2002; Zhao et al. 2010). The region ASA.S1 includes a conserved region named as 'stem 29-1' and a highly variable sequence called diagnostic fragment 3 (DF3) that has around 240 nucleotides and is widely used for *Acanthamoeba* genotyping. It's important to note that the literature frequently refers the region of the gene identified by the amplimer ASA.S1 as the JDP, due to the fact that it is amplified using primers JDP1 and JDP2 (Stothard et al. 1998; Schroeder et al. 2001).

This systematic review proposes to analyze if the frequency of genotypes in the genus *Acanthamoeba* in cases of keratitis is the same in different geographic regions around the world, as well as analyzing the types of sample collection and the methods of identification of *Acanthamoeba* used, given its importance for the development of future specific drugs through better correlation between *in vitro* activity and *in vivo* efficacy, as well as for the improvement of diagnostic techniques, resulting in a better prognosis for patients with keratitis.

Materials and methods

This systematic review has been conducted by searching on the database sources including PubMed, Science Direct, Scielo and Google Scholar for articles in English. No restrictions were placed on study date, therefore, articles were found between 2002 and 2020. The keywords used combined in our search strategy were "*Acanthamoeba*", "keratitis" and "genotype". All studies that estimated the genotypes of *Acanthamoeba* spp. in samples from patients with keratitis all around the world were included in this review.

Studies with samples other than samples from patients with keratitis or paraphernalia of contact lenses from patients with keratitis were excluded. Articles that do not have the country where the samples were collected or the genotype of the isolates were also excluded.

All required data such as number of cases, type of sample collected, genotype, molecular biology method employed, primers used, pairwise sequence identities, species of *Acanthamoeba*, collection country and year of the study were extracted from each of the eligible articles and entered into Microsoft Excel software.

Results

Altogether, 2.934 papers were addressed based on four databases including PubMed, Science Direct, Scielo and Google Scholar. Of these, 97 papers were used in the current study, as they met the previously selected inclusion and exclusion criteria.

Our study collected data on the number of published articles that contained cases of keratitis caused by *Acanthamoeba* spp. and the respective genotypes and year of publication. Thereafter, the number of publications per year can be observed according to the Figure 1, where it's possible to see that the first publication took place in the year 2002 (Booton et al. 2002).

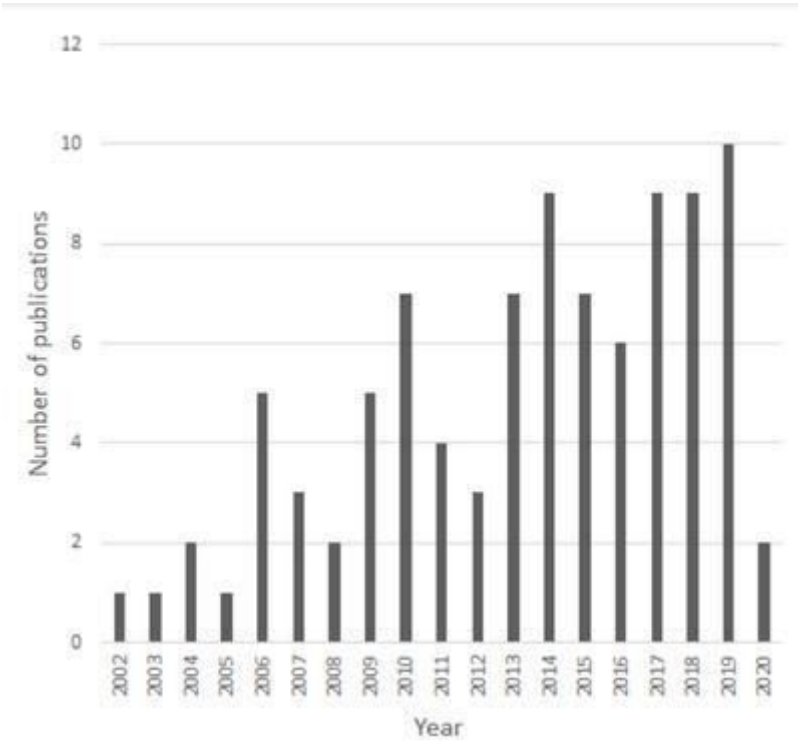

Figure 1: Number of publications per year of cases of *Acanthamoeba* keratitis that indicate the genotype of the isolate.

Regarding to the type of sample collected in each study, it is possible to observe that the most diverse samples were collected. Corneal scrapes were the most collected, followed by contact lens samples. However, most authors used more than one type of sample in their studies, associating corneal scrapings with corneal biopsies, contact lenses, corneal swabs, contact lens paraphernalia (lens maintenance solution, lens case), and these associations can be seen in detail in Table 1. Besides that, unusual samples were collected and used in only one study each, as corneal button (Zhao et al. 2010), vitreous fluid (Khairnar et al. 2011) and amniotic membrane (Sharifi et al. 2010), which is a graft used for treatment of corneal epithelial defects. Some studies did not specify the type of sample collected, using only terms such as ‘’symptomatic keratitis human patients’’ or ‘’corneal samples’’. Six of the studies did not contain information about the sample collected.

Table 1: Details of the samples collected in each study and their respective references

Type of sample collected	References
Corneal scrapings	Sharma et al. 2004; Zhang et al. 2004; Spanakos et al. 2006; Ertabaklar et al. 2007; Xuan et al. 2007; Ozkoc et al. 2008; Booton et al. 2009; Iovieno et al. 2010; Nagyová et al. 2010b; Niyyati et al. 2010; Nuprasert et al. 2010; Kliescikova et al. 2011; Lorenzo-Morales el al. 2011; Takaoka-Sugihara et al. 2012; Arnalich-Montiel et al. 2013b; Duarte et al. 2013; Buerano et al. 2014; Chomicz et al. 2014; Ghamilouie et al. 2014 a; Ghamilouie et al. 2014 b; Chomicz et al. 2015; Koltas et al. 2015; Behera et al. 2016; Padzik et al. 2016; Tawfeek et al. 2016; Baltaza et al. 2017; Padzik et al. 2017; Alves et al. 2018; Baltaza et al. 2018; Buchele et al. 2018; Fabres et al. 2018; Possamai et al. 2018; Taher et al. 2018; Bahreini et al. 2019; Baltaza et al. 2019; Omaña-Molina et al. 2019; Orosz et al. 2019; Tananuvat et al. 2019; Alver et al. 2020; Prithiviraj et al. 2020

Contact lens	Jeong et al. 2007; Omaña-Molina et al. 2013; Heredero-Bermejo et al. 2015; Martín-Pérez et al. 2019
Corneal scrapings, contact lenses	Yera et al. 2008; Dendana et al. 2013; González-Robles et al. 2014; Abedkhojasteh et al. 2015; Hajialilo et al. 2016; Omaña-Molina et al. 2016; Casero et al. 2017; Scheid and Balczun 2017
Corneal scrapings, corneal biopsies	Maghsood et al. 2005; Yera et al. 2007; Gatti et al. 2010; Arnalich-Montiel et al, 2014
Corneal scrapings, contact lenses and contact lens paraphernalia	Booton et al. 2002; Mubareka et al. 2006; Lorenzo-Morales et al. 2007; Niyyati et al. 2009; Antonelli et al. 2018; Esboei et al. 2020
Corneal scrapings and corneal button	Zhao et al. 2010
Corneal scrape, biopsies and/or cotton swabs, contact lenses and contact lens paraphernalia	De Jonckheere 2003; Di Cave et al. 2009; Ledee et al. 2009; Risler et al. 2013; Del Chierico et al. 2016; Chegeni et al. 2019; Jercic et al. 2019
Corneal scrapings and swabs, contact lens and contact lens paraphernalia (lens case, lens solutions)	Abe and Kimata 2010; Di Cave et al. 2014; Wagner et al. 2016
Corneal and eye scrapings, corneal biopsy,	Sharifi et al. 2010; Khairnar et al. 2011

eye and corneal swabs, contact lens and it's solutions, vitreous fluid and amniotic membrane	
Symptomatic keratitis human patients, corneal samples	Paterson et al. 2011; Rahman et al. 2013; Niyyati and Dodanghe 2015; Rocha-Cabrera et al. 2015; Fu-Chin et al. 2017; Martín-Pérez et al. 2017; Nakaminami et al. 2017; Sant'Ana et al. 2017; Li et al. 2019
No information	Arnalich-Montiel et al. 2013a; Mirjalali et al. 2013; Rahman et al. 2013; Nakagawa et al. 2015; Megha et al. 2018; Niyyati et al. 2018

Regarding to the methods used, it is possible to infer that the vast majority of articles used the conventional PCR technique for the identification of *Acanthamoeba* isolates. However, other methodologies have been used, such as Real-time polymerase chain reaction (Nakagawa et al. 2015; Antonelli et al. 2018), RT polymerase chain reaction (RT-PCR) (Fu-Chin et al. 2017), triplex quantitative real-time polymerase chain reaction (qPCR) assay (Scheid and Balczun 2017), PCR-RFLP (Ghamilouie et al. 2014 b), real-time FRET PCR (Orosz et al. 2019), multiplex PCR (Sharma et al. 2004), and real-time fluorescence resonance energy transfer polymerase chain reaction (Orosz et al. 2018). In addition, some studies have associated methodologies, such as conventional PCR and Real-time PCR (Koltas et al. 2015), cDNA-AFLP and real time RT-PCR (Abedkhojasteh et al. 2015), besides conventional PCR e MALDI-TOF (Megha et al. 2018). JDP1 and JDP2 are the most widely used primer set, used in fifty studies. This primer set is utilized for PCR amplification of the

Rns amplicon ASA.S1 of 18S rRNA, which encodes the highly variable DF3 region. However, some studies used different combinations of primers, such as 5′-NTR and VP1 (Orosz et al. 2018; Orosz et al. 2019), CRN5-1137 and E528F-1492R (Martín-Pérez et al. 2017), 892C and JDP2 (Behera et al. 2016), SSU1 and SSU2 (Ertabaklar et al. 2007), YKF2 and JDF2 (Rahman et al. 2013), SSU2F and JDP2 (Risler et al. 2013) and also eukaryote-specific primers CRN5 and 1137, which amplify the GTSA.B1 (Possamai et al. 2018, Nagyová et al. 2010a).

The ideal percentage of similarity between isolates and genotypes sequences of databases is higher than 95%, considering that each genotype is similar, with few differences, we need to be sure of the result. In the studies analyzed, most articles comply with this suggestion. Meanwhile, many articles did not even contain this information and others had similarity values between 95 and 97%.

In the studies analyzed, genotyped cases of keratitis caused by *Acanthamoeba* spp. were found on 4 continents, being Asia, America, Europe and Africa. Genotyped *Acanthamoeba* isolates were found in 31 countries around the world, in 8 countries in Asia, 7 in America, 14 in Europe and 2 in Africa. In addition, two of the studies did not specify the country where the samples were collected, calling them ‘’North America’’ and ‘’Southern Africa’’, referring only to the continent. No studies pertaining to the continent Australia have been found so far.

The total number of genotyped cases of amoebic keratitis caused by *Acanthamoeba* spp. found worldwide is equal to 878. Of these, were found 373 in Asia, 197 in America, 268 in Europe and 40 in Africa. The total number of cases per genotype in each continent can be seen in Figure 2. In this same figure, we can see that the T4 genotype is the most prevalent and Asia is the continent where there are more cases of this genotype.

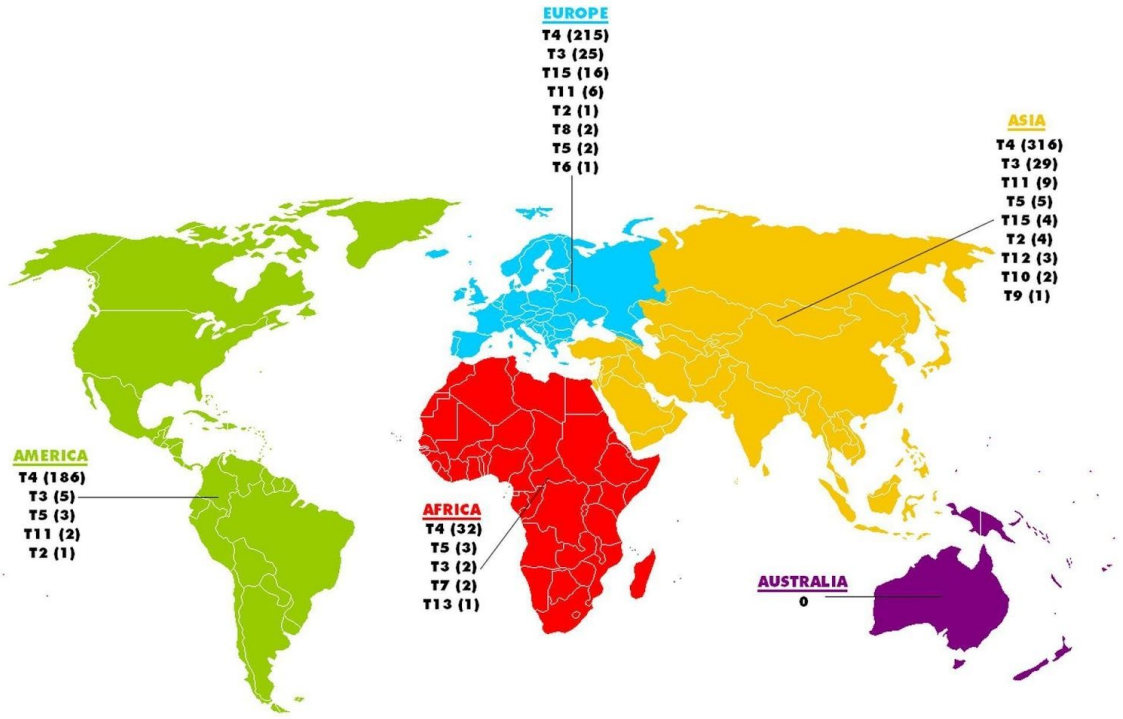

Figure 2: Relationship between cases of amoebic keratitis caused by *Acanthamoeba* spp. with their respective continents and genotypes found. In parentheses, the number of cases of each genotype can be seen.

In Figure 3 it is possible to observe that, undoubtedly, the T4 genotype is the most prevalent worldwide, corresponding to 749 of the total 878 cases. In addition, the prevalence of each genotype and the respective number of cases (in parentheses) were: T3 (61), T15 (20), T11 (17), T5 (13), T2 (6), T12 (3), T7 (2), T8 (2), T10 (2), T6 (1), T9 (1) and T13 (1). Besides that, it's possible to see that genotypes T1, T14, T16, T17, T18, T19, T20, T21 and T22 were not found to cause AK.

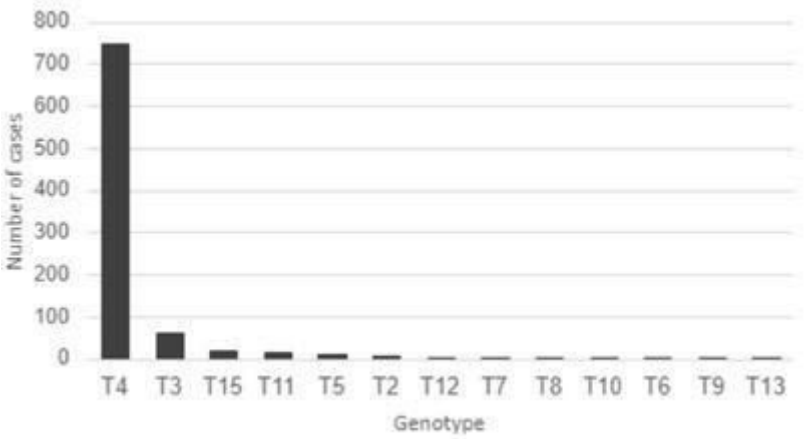

Figure 3: Absolute number of worldwide cases of *Acanthamoeba* keratitis and their respective genotypes.

In more detail, Table 2 shows the number of cases of each genotype in the different countries in each continent and their respective references.

Table 2: Countries where cases of keratitis were found on each continent and their respective genotypes. The number of cases for each genotype can be found in parentheses.

Continent	Genotypes	References
Asia		
China	T4 (55) T3 (2)	Booton et al. 2002; Zhang et al. 2004; Zhao et al. 2010; Li et al. 2019
India	T4 (66), T11 (4), T12 (3), T10 (2), T5 (2), T3 (1)	Sharma et al. 2004; Behera et al. 2016; Megha et al. 2018; Prithiviraj et al. 2020

Iran	T4 (90), T3 (5), T11 (5), T2 (3), T9 (1)	Niyyati et al. 2009; Niyyati et al. 2010; Mirjalali et al. 2013; Ghamilouie et al. 2014a; Ghamilouie et al. 2014b; Abedkhojasteh et al. 2015; Maghsood et al. 2015; Niyyati and Dodanghe 2015; Hajialilo et al. 2016; Niyyati et al. 2018; Bahreini et al. 2019; Chegeni et al. 2019; Esboei et al. 2020
Japan	T4 (61), T3 (6), T5 (2)	Abe and Kimata 2010; Takaoka-Sugihara et al. 2012; Rahman et al. 2013; Nakagawa et al. 2015; Nakaminami et al. 2017
Korea	T4 (9)	Jeong et al. 2007; Xuan et al. 2007
Philippines	T4 (1)	Buerano et al. 2014
Taiwan	T4 (2)	Fu-Chin et al. 2017
Thailand	T4 (5), T5 (1)	Nuprasert et al. 2010; Tananuvat et al. 2019
Turkey	T4 (27), T3 (15), T15 (4),	Ertabaklar et al. 2007;

	T2 (1)	Ozkoc et al. 2008; Koltas et al. 2015; Alver et al. 2020
America		
Argentina	T4 (10)	Casero et al. 2017
Brazil	T4 (22), T3 (1), T5 (1)	Duarte et al. 2013; Sant'Ana et al. 2017; Alves et al. 2018; Buchele et al. 2018; Fabres et al. 2018; Possamai et al. 2018
Canada	T4 (29)	Mubareka et al. 2006; Khairnar et al. 2011
Chile	T4 (73), T11 (2), T2 (1)	Jercic et al. 2019
Mexico	T4 (4), T3 (2)	Omaña-Molina et al. 2013; González-Robles et al. 2014; Omaña-Molina et al. 2016; Omaña-Molina et al. 2019;
United States	T4 (35), T3 (2), T5 (1)	Booton et al. 2009, Ledee et al. 2009
Venezuela	T4 (13)	Wagner et al. 2016
North America	T5 (1)	Iovieno et al. 2010
Europe		

Austria	T6 (1)	Blaschitz et al. 2006
Belgium	T4 (15)	De Jonckheere 2003
Czech Republic	T4 (3), T3 (1)	Nagyová et al. 2010b; Kliescikova et al. 2011
England	T4 (1)	Paterson et al. 2011
France	T4 (33), T3 (3), T2 (1), T5 (1), T11 (1)	Yera et al. 2007; Yera et al. 2008; Risler et al. 2013
Germany	T4 (1)	Scheid and Balczun 2017
Greece	T4 (4), T5 (1)	Spanakos et al. 2006
Hungary	T4 (6), T8 (2)	Orosz et al. 2018; Orosz et al. 2019
Italy	T4 (68), T15 (14), T3 (12), T11 (1)	Di Cave et al. 2009; Del Gatti et al. 2010; Di Cave et al. 2014; Chierico et al. 2016; Antonelli et al. 2018
Poland	T4 (31)	Chomicz et al. 2014; Chomicz et al. 2015; Padzik et al. 2016; Baltaza et al. 2017; Padzik et al. 2017; Baltaza et al. 2018; Baltaza et al. 2019

Slovakia	T4 (3), T15 (1)	Nagyová et al. 2010b
Spain	T4 (40), T3 (8), T11 (3)	Lorenzo-Morales et al. 2007; Lorenzo-Morales el al. 2011; Arnalich-Montiel et al. 2013a; Arnalich-Montiel et al. 2013b; Arnalich-Montiel et al, 2014; Heredero-Bermejo et al. 2015; Rocha-Cabrera et al. 2015; Martín-Pérez et al. 2017; Martín-Pérez et al. 2019
Sweden	T4 (10), T3 (1), T11 (1), T15 (1)	Sharifi et al. 2010
Africa		
Egypt	T4 (27), T5 (3), T3 (2), T7 (2)	Tawfeek et al. 2016; Taher et al. 2018
Tunisia	T4 (5)	Dendana et al. 2013
Southern Africa	T13 (1)	Grün et al. 2014

This systematic review showed that the T4 genotype, the most prevalent, besides being responsible for the largest number of cases worldwide, is also related to a greater number of species of *Acanthamoeba*. In addition, it can be seen in Table 3 that the same species is related to different genotypes. For example, *A. castellani* and *A. palestinensis* belong to genotype T4 and T2 each, *A. culbertsoni* belong to genotype T4 and T10 and *A. hatchetti* belong to genotype T4, T6 and T11. However, it is important to note that most articles do not have the identification of *Acanthamoeba* species.

Table 3: *Acanthamoeba* species related to each genotype and their respective references.

Genotypes	Species	References
T4	*A. castellani*, *A. polyphaga*, *A. palestinensis*, *A. culbertsoni*, *A. triangularis*, *A. rhysodes*, *A. royreba*, *A. quina*, *A. hatchetti*	Maghsood et al. 2005; Spanakos et al. 2006; Ertabaklar et al. 2007; Xuan et al. 2007; Ozkoc et al. 2008; Di Cave et al. 2009; Sharifi et al. 2010; Omaña-Molina et al. 2013; Di Cave et al. 2014; Ghamilouie et al. 2014b; Koltas et al. 2015; Omaña-Molina et al. 2016; Casero et al. 2017; Baltaza et al. 2017, Fu-Chin et al. 2017; Nakaminami et al.

		2017; Padzik et al. 2017; Sant'Ana et al. 2017; Baltaza et al. 2018; Megha et al. 2018; Taher et al. 2018; Baltaza et al. 2019; Omaña-Molina et al. 2019; Prithiviraj et al. 2020
T2	*A. castellani*; *A. palestinensis*	Maghsood et al. 2005; Alver et al. 2020
T3	*A. griffini*	Maghsood et al. 2005; Sharifi et al. 2010; Di Cave et al. 2014; González-Robles et al. 2014; Heredero-Bermejo et al. 2015; Koltas et al. 2015; Omaña-Molina et al. 2016; Megha et al. 2018; Taher et al. 2018; Martín-Pérez et al. 2019
T5	*A. lenticulata*	Spanakos et al. 2006; Ledee et al. 2009; Iovieno et al. 2010; Rahman et al. 2013; Megha et al. 2018; Taher et al. 2018

T6	*A. hatchetti*	Blaschitz et al. 2006, Megha et al. 2018
T7	*A. astronyxis*	Tawfeek et al. 2016
T11	A. *stevensoni*, *A. hatchetti*	Sharifi et al. 2010; Lorenzo-Morales el al. 2011; Hajialilo et al. 2016; Prithiviraj et al. 2020
T10	*A. culbertsoni*	Behera et al. 2016
T15	*A. jacobsi*	Di Cave et al. 2009; Sharifi et al. 2010; Koltas et al. 2015; Di Cave et al. 2014

Discussion

As a result of their ubiquity, the cosmopolitan protozoa of the genus *Acanthamoeba* pose a risk to human health, due to their ability of being inside the host or in the environment. Studies are still needed in order to elucidate the pathogenesis of AK and other diseases caused by this free-living amoeba, for improvement of diagnostic techniques and medications with specific therapeutic targets.

Our study shows that in the last 20 years there has occurred a crescent increase in the number of articles containing the genotyping of *Acanthamoeba* isolates causing keratitis, especially in the last decade. Nevertheless, unfortunately the pandemic of Sars-Cov-2 caused the number of publications to decrease almost as it did twenty years ago, which is obviously an exception to the upward trend. Several factors are related to the increase in the number of

AK cases worldwide every year, such as the widespread use of contact lenses for vision correction or cosmetic purposes and better diagnostics, therefore becoming an emerging disease (Panjwan 2010; Astorga et al. 2011). However, we believe that the number of cases is still underreported and, in addition, not all diagnosed cases are genotyped. Although there are several studies with genotyping in certain countries, to the best of our knowledgment did not exist until the present moment a single study that listed all of these cases, thus providing a worldwide idea of the frequency of these genotypes in AK.

Various sample collection methods have been shown to be effective for the isolation of *Acanthamoeba* spp. causing keratitis, such as corneal scraping, corneal biopsies and corneal smears, as well as collections of contact lenses and their accessories, such as lens cases and solutions for lenses. However, our study shows that the most chosen samples for the isolation of *Acanthamoeba* were the corneal scrape and contact lenses of patients with keratitis.

Although our review shows that several different techniques can be used for the diagnosis of *Acanthamoeba*, it also shows that a very useful molecular technique used for the detection and genotyping of *Acanthamoeba* spp. is the Polymerase Chain Reaction (PCR) and that this is the methodology used in the vast majority of studies, with rare exceptions. The method is usually performed through amplification of the fragment of the 18S rRNA gene using the JDP1 (5'-GGCCCAGATCGTTTACCGTGAA-3') and JDP2 (5'-TCTCACAAGCTGCTAGGGAGTCA-3') primers (Schroeder et al. 2001; Lorenzo-Morales et al. 2015).

Nevertheless, other sets of PCR primers would also provide genotype identification, since the amplimer ASA.S1 did not appear to distinguish between all sequence types. The genotype specific amplimer B1 (GTSA.B1) has proven to be accurated in identifying the

different genotypes (Fuerst et al. 2015), having been chosen in two studies (Nagyová et al. 2010b; Possamai et al. 2018).

The detection of *Acanthamoeba* genotypes in keratitis patients can also be performed using real-time PCR assay. This method allows an accurate and rapid diagnosis (Visvesvara et al. 2007; Corsaro et al. 2015; Maycock and Jayaswal 2016), then several studies have also reported its use alone (Nakagawa et al. 2015; Fu-Chin et al. 2017; Antonelli et al. 2018) or in association with other methodologies, as the conventional PCR (Koltas et al. 2015) or cDNA-AFLP (Abedkhojasteh et al. 2015). Furthermore, one study (Sharma et al. 2004) used a multiplex real-time PCR assay, that has been newly used and developed for the detection of FLA. In addition to enabling simultaneous detection of *Acanthamoeba* and other FLA of the genres *Naegleria* and *Balamuthia* in the same human specimen, it also allows the detection of 10 different genotypes of *Acanthamoeba* at the same time, which could facilitate the laboratory routine in genotyping isolates (Qvarnstrom et al. 2006; Goldschmidt et al. 2009). Another method used in one article (Ghamilouie et al. 2014b) that has been shown to be effective and sensitive is the Restriction Fragment Length Polymorphism (RFLP), which is the phylogenetic analysis of mitochondrial DNA, allowing understanding about the relationships among different *Acanthamoeba* strains (Kong et al. 2002; Schuster and Visvesvara 2004; Fuerst et al. 2015). In the last years, a method called Matrix-assisted Laser Desorption-ionization Time-of-flight MS (MALDI-TOF MS) has been improved for different microorganisms including protists. It has been used not only for identifying but also to establish strain differences based on biomarkers fingerprints that are characteristic protein patterns. Among the advantages, it is possible to mention that it is a practical and rapid method, enabling identification of amoeba in 15 minutes (Moura et al. 2003a; Moura et al. 2003b). However, this methodology still needs to be improved for the diagnosis of

Acanthamoeba, because there are still divergences between the results obtained by it and by conventional PCR (Megha et al. 2018).

Our study shows that, when analyzing all genotyped cases of AK, there are 749 cases genotyped as T4, which corresponds to 85,31% of the total number of cases reported worldwide. In addition, the T4 genotype was the most prevalent on all continents where cases of keratitis were found, which suggests that even in different countries, isolates with this genotype may have similar pathogenic properties. It is known that an important initial step in the pathogenesis of AK is the adherence to corneal epithelial cells that is strongly related to expression of mannose binding protein. This mannose binding protein in T4 genotype appears to bind more tightly to the membrane surface of host cells, making this genotype more cytotoxic than others, culminating in a greater number of infections (Hurt et al. 2003; Garate et al. 2006; Ledee et al. 2009; Noorjahan 2010). Besides that, exposure to mannose generates liberation of a low molecular weight protease called MIP133, which has the ability to cause cytolytic effect to corneal epithelial cells (Hurt et al. 2003; Garate et al. 2006; Ledee et al. 2009; Noorjahan 2010). As a result, it is suggested that T4 is the most virulent genotype and owner of properties that enhance its transmissibility, given its greatest environmental distribution (Maghsood et al. 2005; Ledee et al. 2009). Therefore, the highest mannose binding protein expression in T4 genotype could be an effective specific target for new therapeutic approaches that would serve as a treatment for the vast majority of cases of amoebic keratitis.

In addition to T4, our study shows that the second most prevalent genotype is T3, with 61 reported cases, followed by T15 with 20 cases, T11 with 17 and T5 with 13. Other genotypes were also reported as keratitis causing, although less frequently, as T2 (6 cases), T12 (3 cases), T7, T8 and T10 (2 cases each). In addition, the T6, T9 and T13 genotypes also caused *Acanthamoeba* keratitis, with one case each. It is also possible to observe that the

distribution of frequencies between genotypes in the four continents where cases of AK were found is very similar. Related to T3, it was the second most prevalent genotype in three of the four continents where the AK cases were reported. The exception occurred in Africa, where the T5 genotype was the second most prevalent. However, it is important to note that the diagnosis of AK is difficult, and the vast majority of diagnosed cases are not genotyped. Furthermore, our study clearly showed that there is a difference in the number of genotyped cases between the continents, with the African continent having the lowest number of cases and Asia the largest, probably on account of financial resources. Even so, the genotypic distribution of cases reported worldwide so far has been described in our study.

Although the T4 genotype is the most prevalent, genotyping of all isolates from cases of *Acanthamoeba* keratitis is essential, due to the fact that scientific literature shows that infections caused by non-T4 genotypes are more aggressive. Besides, the outcomes are extremely unfavorable (Iovieno et al. 2010; Sharifi et al. 2010). Moreover, more resistance to multipurpose contact lens cleaning solutions are related to non-T4 genotypes, as T3 and T5 (Shoff et al. 2007), besides a worse response to medical therapy, longer delays to diagnosis when compared with T4 genotype and greater need for surgical intervention, that is, worse clinical outcomes (Arnalich-Montiel et al. 2014).

Our study made it possible to observe that although *A. castellani* and *A. polyphaga* be the species whose genotype is T4 in most isolates, other species were part of this genotype, such as *A. culbertsoni*, *A. triangularis*, *A. rhysodes*, *A. royreba*, *A. quina*, and *A. hatchetti*. In addition, *A. castellani* and *A. palestinensis* were also related to T2 genotype and the T3 genotype were only related to *A. griffini*. That is, the classification of *Acanthamoeba* in species and genotypes still needs to be improved, so that these relationships are more clearly understood. Besides that, we agree with a recent study (Corsaro 2020), which says that the identification of *Acanthamoeba* would currently have a clearer organization, due to the fact

that the classification using only morphology was once appropriate, but today it is cause for confusion, relating several species to the same genotype.

It is also important to mention that there is still no single effective treatment that can be used in cases of AK, so novel therapeutics are needed in order to totally eliminate both amoeba life forms, that is, trophozoites and cysts. It is important to remember that cysts are very resistant and related to the recurrence of infection. Given the differences in pathogenicity, virulence, susceptibility to treatment and clinical outcomes between genotypes, genotyping is a path to be taken so that we have a better correlation between *in vitro* and *in vivo* efficacies, resulting in better drug therapies and successful treatment in AK cases.

In conclusion, *Acanthamoeba* genotyping is very important not only for taxonomic purposes and understanding the geographical distribution of species, but also for clinical and epidemiological studies, understanding the pathogenesis and clinical outcomes of this infection. The scientific literature shows that non-T4 genotypes produce worse symptoms and have poorer response to medical therapy than genotype T4 (Arnalich-Montiel et al. 2014), although more than 85% of *Acanthamoeba* keratitis cases have been linked with T4 genotype, which is why it's supposedly the most virulent. AK remains a challenging disease to diagnose and treat, so further studies should be conducted in order to elucidate what makes some genotypes more pathogenic than others. This information would also play a fundamental role in providing more reliable diagnosis and novel therapeutic strategies.

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Conflict of interest

The authors declare that they have no conflict of interest.

References:

Abedkhojasteh H, Niyyati M, Rezaei S, Mohebali M, Farnia S, Kazemi-Rad E, Roozafzoon R, Sianati H, Rezaeian M, Heidari M (2015) Identifying differentially expressed genes in trophozoites and cysts of *Acanthamoeba* T4 genotype: Implications for developing new

treatments for *Acanthamoeba* keratitis. Eur J Protistol 51(1):34-41. https://doi.org/10.1016/j.ejop.2014.10.001

Abe N, Kimata I (2010) Genotyping of *Acanthamoeba* isolates from corneal scrapings and contact lens cases of *Acanthamoeba* keratitis patients in Osaka, Japan. Jpn J Infect Dis 63(4):299-301.

Alver O, Baykara M, YÜrÜk M, TÜzemen NÜ (2020) *Acanthamoeba* Keratitis and *Acanthamoeba* Conjunctivitis: A Case Report. Iran J Parasitol 15(2):272-277.

Alves DSMM, Gonçalves GS, Moraes AS, Alves LM, Neto JRC, Hecht MM, Nitz N, Gurgel-Gonçalves R, Bernardes G, Castro AM, Chalita MR, Vinaud MC (2018) The first *Acanthamoeba* keratitis case in the Midwest region of Brazil: diagnosis, genotyping of the parasite and disease outcome. Rev Soc Bras Med Trop 51(5):716-719. https://doi.org/10.1590/0037-8682-0010-2018

Antonelli A, Favuzza E, Galano A, Di Filippo MM, Ciccone N, Berrilli F, Mencucci R, Di Cave D, Rossolini GM (2018) Regional spread of contact lens-related *Acanthamoeba* keratitis infection in Italy. New Microbiologica 41(1):83-85.

Arnalich-Montiel F, Jaumandreu L, Leal M, Valladares B, Lorenzo-Morales J (2013a) Scleral and Intraocular Amoebic Dissemination in *Acanthamoeba* Keratitis. Cornea 32(12):1625-1627. https://doi.org/10.1097/ICO.0b013e31829ded51

Arnalich-Montiel F, Lorenzo-Morales J, Irigoyen C, Morcillo-Laiz R, López-Vélez R, Muñoz-Negrete F, Piñero JE, Valladares B (2013b) Co-isolation of *Vahlkampfia* and

Acanthamoeba in *Acanthamoeba*-like keratitis in a Spanish population. Cornea 32(5):608-614. https://doi.org/10.1097/ICO.0b013e31825697e6

Arnalich-Montiel F, Lumbreras-Fernández B, Martín-Navarro CM, Valladares B, Lopez-Velez R, Morcillo-Laiz R, Lorenzo-Morales J (2014) Influence of *Acanthamoeba* Genotype on Clinical Course and Outcomes for Patients with *Acanthamoeba* Keratitis in Spain. Journal of Clinical Microbiology 52:1213–1216. https://doi.org/10.1128/jcm.00031-14

Astorga B, Lorenzo‐Morales J, Martín‐Navarro CM, Alarcón V, Moreno J, González AC, Navarrete E, Piñero JE, Valladares B (2011) *Acanthamoeba* belonging to T3, T4, and T11: genotypes isolated from air-conditioning units in Santiago, Chile. J Eukaryot Microbiol 58:542–544. https://doi.org/10.1111/j.1550-7408.2011.00584.x

Bahreini MS, Motazedian MH, Bamdad S, Afshar MJA, Asgari QQ (2019) Detection of Free Living Amoeba Infection in Patients with Suspected Central Nervous System and Keratitis Disease in Shiraz, Southern Iran. J Health Sci Surveillance 7:2. https://doi.org/10.30476/JHSSS.2020.84999.1052

Baltaza W, Padzik M, Szaflik JP, Dybicz M, Grochowska A, Kuryłowicz A, Chomicz L (2018) Comparative examination on selected amphizoic amoebae in terms of their in vitro temperature tolerance – a possible indirect marker of potential pathogenicity of *Acanthamoeba* strains. Annals of Parasitology 64(4):317–322. https://doi.org/10.17420/ap6404.166

Baltaza W, Padzik M, Szaflik JP, Dybicz M, Hendiger1 E, Chomicz L (2017) Amoebicidal or

amoebostatic influence of disinfectants used in health facilities and laboratories on corneal strains of *Acanthamoeba*. Annals of Parasitology 63(3):167–172. https://doi.org/10.17420/ap6303.102

Baltaza W, Padzik M, Szaflik JP, Perkowski K, Dybicz M, Chomicz L (2019) Evaluation of in vitro effects of selected physical and chemical agents on detected in Poland *Acanthamoeba* strains – factors of increasing threats for public health. Ann Parasitol 65(1):19-25. https://doi.org/10.17420/ap6501.179

Behera HS, Panda A, Satpathy G, Bandivadekar P, Vanathi M, Agarwal T, Nayak N, Tandon R (2016) Genotyping of *Acanthamoeba* spp. and characterization of the prevalent T4 type along with T10 and unassigned genotypes from amoebic keratitis patients in India. J Med Microbiol 65(5):370-376. https://doi.org/10.1099/jmm.0.000234

Berger P, Papazian L, Drancourt M, La Scola B, Auffray JP, Raoult D (2006) Ameba-associated microorganisms and diagnosis of nosocomial pneumonia. Emerg Infect Dis 12:248–255. https://doi.org/10.3201/eid1202.050434

Blaschitz M, Köhsler M, Aspöck H, Walochnik J (2006) Detection of a serine proteinase gene in *Acanthamoeba* genotype T6 (Amoebozoa: Lobosea). Exp Parasitol 114(1):26-33. https://doi.org/10.1016/j.exppara.2006.02.004

Booton GC, Joslin CE, Shoff M, Tu EY, Kelly DJ, Fuerst PA (2009) Genotypic identification of *Acanthamoeba* sp. isolates associated with an outbreak of *Acanthamoeba* keratitis. Cornea 28(6):673–676. https://doi.org/10.1097/ICO.0b013e31819342a7

Booton GC, Kelly DJ, Chu YW, Seal DV, Houang E, Lam DS, Byers TJ, Fuerst P A (2002) 18S ribosomal DNA typing and tracking of *Acanthamoeba* species isolates from corneal scrape specimens, contact lenses, lens cases, and home water supplies of *Acanthamoeba* keratitis patients in Hong Kong. J Clin Microbiol 40:1621–1625. https://doi.org/10.1128/jcm.40.5.1621-1625.2002

Booton GC, Rogerson A, Bonilla TD, Seal DV, Kelly DJ, Beattie TK, Tomlinson A, Lares-Villa F, Fuerst PA, Byers TJ (2004) Molecular and physiological evaluation of subtropical environmental isolates of *Acanthamoeba* spp., causal agent of *Acanthamoeba* keratitis. J Eukaryot Microbiol 51(2):192-200. https://doi.org/10.1111/j.1550-7408.2004.tb00545.x

Buchele MLC, Wopereis DB, Casara F, Macedo JP, Rott MB, Monteiro FBF, Bazzo ML, Spada FR, Santos JI, Caumo KS (2018) Contact lens-related polymicrobial keratitis: *Acanthamoeba* spp. genotype T4 and *Candida albicans*. Parasitology Research 117:3431–3436. https://doi.org/10.1007/s00436-018-6037-x

Buerano CC, Trinidad AD, Fajardo LS, Cua IY, Baclig MO, Natividad FF (2014) Isolation of acanthamoeba genotype t4 from a non-contact lens wearer from the Philippines. Trop Med Health 42(4):145-147. https://doi.org/10.2149/tmh.2014-15

Casero RD, Mongi F, Laconte L, Rivero F, Sastre D, Teherán A, Herrera G, Ramírez JD (2017) Molecular and morphological characterization of *Acanthamoeba* isolated from corneal scrapes and contact lens wearers in Argentina. Infect Genet Evol 54:170-175.

https://doi.org/10.1016/j.meegid.2017.06.031

Castrillón JC, Orozco LP (2013) *Acanthamoeba* spp. como parásitos patógenos y oportunistas. Rev Chilena Infectol 30(2):147-155. http://dx.doi.org/10.4067/S0716-10182013000200005

Chegeni TN, Ghaffarifar F, Pirestani M, Khoshzaban F, Dalimi A, Maspi N (2019) Genotyping of *Acanthamoeba* Species Isolated from Keratitis Patients by PCR Sequencing Methods in Tehran, Iran. International Journal of Medical Laboratory 6(4):259-265. https://doi.org/10.18502%2Fijml.v6i4.2002

Chomicz L, Conn DB, Padzik M, Szaflik JP, Walochnik J, Zawadzki PJ, Pawlowski W, Dybicz M (2015) Emerging Threats for Human Health in Poland: Pathogenic Isolates from Drug Resistant *Acanthamoeba* Keratitis Monitored in terms of Their *In Vitro* Dynamics and Temperature Adaptability. Biomed Res Int 2015:231285. https://doi.org/10.1155/2015/231285

Chomicz L, Padzik M, Szaflik JP, Nahorski WL, Kryczka T, Szaflik J (2014) Monitoring of in vitro dynamics of *Acanthamoeba* strains isolated from infected eyes as a useful tool in keratitis management. Experimental Parasitology 145:73-77. https://doi.org/10.1016/j.exppara.2014.06.021.

Clarke DW, Niederkorn JY (2006) The pathophysiology of *Acanthamoeba* keratitis. Trends Parasitol 22:175–180. https://doi.org/10.1016/j.pt.2006.02.004

Corsaro D, Köhsler M, Filippo MM, Venditti D, Monno R, Di Cave D, Berrilli F, Walochnik J (2017) Update on *Acanthamoeba jacobsi* genotype T15, including full-length 18S rDNA molecular phylogeny. Parasitol Res 116:1273–84. https://doi.org/10.1007/s00436-017-5406-1

Corsaro D (2020) Update on *Acanthamoeba* phylogeny. Parasitol Res 119:3327–3338. https://doi.org/10.1007/s00436-020-06843-9

Corsaro D, Walochnik J, Köhsler M, Rott MB (2015) *Acanthamoeba* misidentification and multiple labels: Redefining genotypes T16, T19, and T20 and proposal for *Acanthamoeba micheli* sp. nov. (genotype T19). Parasitol Res 114:2481–2490. https://doi.org/10.1007/s00436-015-4445-8

Costa AO, Castro EA, Ferreira GA, Furst C, Crozeta MA, Thomaz-Soccol V (2010) Characterization of *Acanthamoeba* isolates from dust of a public hospital in Curitiba, Paraná, Brazil. J Eukaryot Microbiol 57:70–75. https://doi.org/10.1111/j.1550-7408.2009.00453.x.

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

De Jonckheere JF (2003) Epidemiological typing of *Acanthamoeba* strains isolated from keratitis cases in Belgium. Bull Soc Belge Ophtalmol 287:27-33.

Del Chierico F, Di Cave D, Accardi C, Santoro M, Masotti A, D'Alfonso R, Berrilli F, Urbani A, Putignani L (2016) Identification and typing of free-living *Acanthamoeba* spp. by MALDI-TOF MS Biotyper. Exp Parasitol 170:82-89.

https://doi.org/10.1016/j.exppara.2016.09.007

Dendana F, Sellami H, Trabelsi H, Neji S, Cheikhrouhou F, Makni F, Ayadi A (2013) *Acanthamoeba* T4 genotype associated with keratitis infections in Tunisia. Parasitol Res 112(1):401-405. https://doi.org/10.1007/s00436-012-3149-6

Derda M, Wojtkowiak-Giera A, Kolasa-Wołosiuk A, Kosik-Bogacka D, Hadaś E, Jagodziński PP, Wandurska-Nowak E (2016) *Acanthamoeba* infection in lungs of mice expressed by toll-like receptors (TLR2 and TLR4). Experimental Parasitology 165:30-34. https://doi.org/10.1016/j.exppara.2016.02.012

Diaz JH (2010) Increasing intracerebral infections caused by free-living amebae in the United States and Worldwide. Journal of Neuroparasitology 1:104. https://doi.org/10.4303/jnp/N100801

Di Cave D, D' Alfonso R, Comlavi KAD, D' Orazi C, Monno R, Berrilli F (2014) Genotypic heterogeneity based on 18S-rRNA gene sequences among *Acanthamoeba* isolates from clinical samples in Italy. Exp Parasitol 145Suppl:S46-S49. https://doi.org/0.1016/j.exppara.2014.05.009

Di Cave D, Monno R, Bottalico P, Guerriero S, D'Amelio S, D'Orazi C, Berrilli F (2009) *Acanthamoeba* T4 and T15 genotypes associated with keratitis infections in Italy. Eur J Clin Microbiol Infect Dis 28(6):607-612. https://doi.org/10.1007/s10096-008-0682-4

Duarte JL, Furst C, Klisiowicz DR, Klassen G, Costa AO (2013) Morphological, genotypic, and physiological characterization of *Acanthamoeba* isolates from keratitis patients and the domestic environment in Vitoria, Espírito Santo, Brazil. Exp Parasitol 135(1):9-14. https://doi.org/10.1016/j.exppara.2013.05.013

Ertabaklar H, Türk M, Dayanir V, Ertuğ S, Walochnik J (2007) *Acanthamoeba* keratitis due to *Acanthamoeba* genotype T4 in a non-contact-lens wearer in Turkey. Parasitol Res 100(2):241-246. https://doi.org/10.1007/s00436-006-0274-0

Esboei BR, Fakhar M, Saberi R, Baratif M, Moslemig M, Hassannia H, Moghadame Y, Jalalloua N (2020) Genotyping and phylogenic study of *Acanthamoeba* isolates from human keratitis and swimming pool water samples in Iran. Parasite Epidemiol Control 11:00164. https://doi.org/10.1016/j.parepi.2020.e00164

Fabres LF, Maschio VJ, Santos DLD, Kwitko S, Ruschel Marinho DR, Araújo BS, Locatelli CI, Rott MB (2018) Virulent T4 *Acanthamoeba* causing keratitis in a patient after swimming while wearing contact lenses in Southern Brazil. Acta Parasitol 63(2):428-432. https://doi.org/10.1515/ap-2018-0050

Fraser MN, Wong Q, Shah L, Holland SP, Morshed M, Isaac-Renton J, Mei Chong, Kibsey P, Patrick DM (2012) Characteristics of an *Acanthamoeba* keratitis outbreak in British Columbia between 2003 and 2007. Ophthalmology 119:1120–1125. https://doi.org/10.1016/j.ophtha.2011.12.041

Fu-Chin H, Min-Hsiu S, Kai-Fei C, Jian-Ming H, Jyh-Wei S, Wei-Chen L (2017)

Characterizing clinical isolates of *Acanthamoeba castellanii* with high resistance to polyhexamethylene biguanide in Taiwan. Journal of Microbiology, Immunology and Infection 50:570-577. https://doi.org/10.1016/j.jmii.2015.10.011

Fuerst PA, Booton GC, Crary M (2015) Phylogenetic analysis and the evolution of the 18S rRNA gene typing system of *Acanthamoeba*. J Eukaryot Microbiol. 62:69–84. https://doi.org/10.1111/jeu.12186.

Garate M, Marchant J, Cubillos I, Cao Z, Khan NA, Panjwani N (2006*) In vitro* pathogenicity of *Acanthamoeba* is associated with the expression of the mannose-binding protein. Invest Ophthalmol Vis Sci 47:1056–1062. https://doi.org/10.1167/iovs.05-0477

Gatti S, Rama P, Matuska S, Berrilli F, Cavallero A, Carletti S, Bruno A, Maserati R, Di Cave D (2010) Isolation and genotyping of *Acanthamoeba* strains from corneal infections in Italy. J Med Microbiol 59:1324-1330. https://doi.org/10.1099/jmm.0.019786-0

Ghamilouie MM, Valadkhani Z, Khoshzaban F (2014) Species Identification of *Acanthamoeba* Strains Isolated from Patients Referring to Farabi Eye Reference Center Using PCR-RFLP Method. J Med Microbiol Infec Dis 2(3):125-129.

Ghamilouie MM, Valadkhani Z, Rahimi F, Khoshzaban F, Aghighi Z, Hassan N (2014) Isolation and Genotyping of *Acanthamoeba* Strains from Corneal Scraps. Iranian Journal of Ophthalmology 26(2):97-101

Gokhale NS (2008) Medical management approach to infectious keratitis. Indian J

Ophthalmol 56:215–220. https://doi.org/10.4103/0301-4738.40360

Goldschmidt P, Degorge S, Benallaoua D, Saint-Jean C, Batellier L, Alouch C, Laroche L, Chaumeil C (2009) New tool for the simultaneous detection of 10 different genotypes of *Acanthamoeba* available from the American Type Culture Collection. Br J Ophthalmol 93:1096-100. https://dx.doi.org/10.1136/bjo.2008.144873

González-Robles A, Salazar-Villatoro L, Omaña-Molina M, Reyes-Batlle M, Martín-Navarro CM, Lorenzo-Morales J (2014) Morphological Features and In Vitro Cytopathic Effect of *Acanthamoeba griffini* Trophozoites Isolated from a Clinical Case. Journal of Parasitology Research 2014:10. http://dx.doi.org/10.1155/2014/256310

Greub G, Raoult D (2004) Microorganisms resistant to free-living amoebae. Clin Microbiol Rev 17:413–433. https://doi.org/10.1128/cmr.17.2.413-433.2004

Grün AL, Stemplewitz B, Scheid P (2014) First report of an *Acanthamoeba* genotype T13 isolate as etiological agent of a keratitis in humans. Parasitol Res 113(6):2395-2400. https://doi.org/10.1007/s00436-014-3918-5

Hajialilo E, Behnia M, Tarighi F, Niyyati M, Rezaeian M (2016) Isolation and genotyping of *Acanthamoeba* strains (T4, T9, and T11) from amoebic keratitis patients in Iran. Parasitol Res. 115(8):3147-3151. https://doi.org/10.1007/s00436-016-5072-8

Hammersmith KM (2006) Diagnosis and management of *Acanthamoeba* keratitis. Curr Opin Ophthalmol 17:327–331. https://doi.org/10.1097/01.icu.0000233949.56229.7d

Heredero-Bermejo I, Criado-Fornelio A, De Fuentes I, Soliveri J, Copa-Patiño JL, Pérez-Serrano J (2015) Characterization of a human-pathogenic *Acanthamoeba griffini* isolated from a contact lens-wearing keratitis patient in Spain. Parasitology 142(2):363-373. https://doi.org/10.1017/S0031182014001140

Hurt M, Neelam S, Niederkorn J, Alizadeh H (2003) Pathogenic *Acanthamoeba* spp. secrete a mannose-induced cytolytic protein that correlates with the ability to cause disease. Infect Immun 71: 6243–6255. https://doi.org/10.1128/IAI.71.11.6243-6255.2003

Iovieno A, Oechslerb RA, Ledee DR, Miller D, Alfonso EC (2010) Drug-resistant severe *Acanthamoeba* keratitis caused by rare T5 *Acanthamoeba* genotype. Eye Contact Lens 36:183–184. https://doi.org/10.1097/icl.0b013e3181da2350

Jeong HJ, Lee SJ, Kim JH, Xuan YH, Hee Lee KH, Park SK, Choi SH, Chung D, Kong HH, Ock MS, Yu HS (2007) *Acanthamoeba*: keratopathogenicity of isolates from domestic tap water in Korea. Exp Parasitol 117(4):357-367. https://doi.org/10.1016/j.exppara.2007.05.003

Jercic MI, Aguayo C, Saldarriaga-Córdoba M, Muiño L, Chenet SM, Lagos J, Osuna A, Fernández J (2019) Genotypic diversity of *Acanthamoeba* strains isolated from Chilean patients with *Acanthamoeba* keratitis. Parasites & Vectors 12:58. https://doi.org/10.1186/s13071-019-3302-5

Jiang C, Sun X, Wang Z, Zhang Y (2006) *Acanthamoeba* keratitis: clinical characteristics and management. Ophthalmology 113:412–416. https://doi.org/10.1016/j.jtos.2015.01.002

Juárez MM, Tártara LI, Cida AG, Reald JP, Bermúdeza JM, Rajala VB, Palmad SD (2018) *Acanthamoeba* in the eye, can the parasite hide even more? Latest developments on the disease. Contact Lens and Anterior Eye 41:245–251. https://doi.org/10.1016/j.clae.2017.12.017

Khairnar K, Tamber GS, Ralevski F, Pillai DR (2011) Comparison of molecular diagnostic methods for the detection of *Acanthamoeba* spp. from clinical specimens submitted for keratitis. Diagn Microbiol Infect Dis 70(4):499-506. https://doi.org/10.1016/j.diagmicrobio.2011.03.019

Khan NA (2006) *Acanthamoeba*: biology and increasing importance in human health. FEMS Microbiology Reviews 30:564-595. https://doi.org/10.1111/j.1574-6976.2006.00023.x

Khan NA, Jarroll EL, Paget TA (2002) Molecular and physiological differentiation between pathogenic and nonpathogenic *Acanthamoeba*. Curr Microbiol 45:197–202. https://doi.org/10.1007/s00284-001-0108-3

Khan NA, Tareen NK (2003) Genotypic, phenotypic, biochemical, physiological and pathogenicity-based categorisation of *Acanthamoeba* strains. Folia Parasitologica 50:97-104. https://doi.org/10.14411/fp.2003.017

Khezri A, Fallah E, Mostafazadeh M, Spotin A, Shahbazi A, Mahami-Oskouei M, Hazratian T (2016) Molecular and morphometric characterization of *Acanthamoeba* spp. from different

water sources of northwest Iran as a neglected focus, co-bordeed with the country of Iraq. Jundishapur Journal of Microbiology 9:38481. https://doi.org/10.5812/jjm.38481

Kilvington S, Gray T, Dart J, Morlet N, Beeching JR, Frazer DG, Matheson M (2004) *Acanthamoeba* keratitis: the role of domestic tap water contamination in the United Kingdom. Invest Ophthalmol Vis Sci 45:165–169. https://doi.org/10.1167/iovs.03-0559

Kitzmann AS, Goins KM, Sutphin JE, Wagoner MD (2009) Keratoplasty for treatment of *Acanthamoeba* keratitis. Ophthalmology 116:864–869. https://doi.org/10.1016/j.ophtha.2008.12.029

Kliescikova J, Kulda J, Nohynkova E (2011) Stress-Induced Pseudocyst Formation - A Newly Identified Mechanism of Protection against Organic Solvents in Acanthamoebae of the T4 Genotype. Protist 162:58-69. https://doi.org/10.1016/j.protis.2010.03.006

Kłopocka W, Rędowicz MJ, Wasik A (2009) Regulation of cortical cytoskeleton dynamics during migration of free-living amoebae. Postępy Biochemii 55:129-137.

Koltas IS, Eroglu F, Erdem E, Yagmur M, Tanır F. (2015) The role of domestic tap water on *Acanthamoeba* keratitis in non-contact lens wearers and validation of laboratory methods. Parasitol Res 114(9):3283-3289. https://doi.org/10.1007/s00436-015-4549-1

Kong HH, Shin JY, Yu HS, Kim J, Hahn TW, Hahn YH, Chung D (2002) Mitochondrial DNA restriction fragment length polymorphism (RFLP) and 18S small-subunit ribosomal DNA PCR-RFLP analyses of *Acanthamoeba* isolated from contact lens storage cases of

residents in southwestern Korea. J Clin Microbiol 40:1199-206. https://doi.org/10.1128/jcm.40.4.1199-1206.2002

Koshler M, Leitner B, Blaschitz M, Michel R, Aspock H, Walochnik J (2006) ITS1 sequence variabilities correlate with 18S rDNA sequence types in the genus *Acanthamoeba* (Protozoa: Amoebozoa). Parasitol Res 98:86–93. https://doi.org/10.1007/s00436-005-0022-x

Kot K, Łanocha-Arendarczyk NA, Kosik-Bogacka DI (2018) Amoebas from the genus *Acanthamoeba* and their pathogenic properties. Annals of Parasitology 64(4):299–308. https://doi.org/10.17420/ap6404.164

Król-Turmińska K, Olender A (2017) Human infections caused by free-living amoebae. Annals of Agricultural and Environmental Medicine 24:254-260. https://doi.org/10.5604/12321966.1233568

Kumar R, Lloyd D (2002) Recent advances in the treatment of *Acanthamoeba* keratitis. Clin Infect Dis 35:434–441. https://doi.org/10.1086/341487

Lanocha N, Kosik-Bogacka D, Maciejewska A, Sawczuk M, Wilk A, Kuźna-Grygiel W (2009) The occurence *Acanthamoeba* (free living amoeba) in environmental and respiratory samples in Poland. Acta Protozoologica 48:271-279.

Lass A, Guerrero M, Li X, Karanis G, Ma L, Karanis P (2017) Detection of *Acanthamoeba* spp. in water samples collected from natural water reservoirs, sewages, and pharmaceutical

factory drains using LAMP and PCR in China. Sci Total Environ 584:489–494. https://doi.org/10.1016/j.scitotenv.2017.01.046

Ledee DR, Booton GC, Awwad MH, Sharma S, Aggarwal RK, Nisz IA, Markus MB, Fuerst PA, Byers TJ (2003) Advantages of using mitochondrial 16S rDNA sequences to classify clinical isolates of *Acanthamoeba*. Invest Ophthalmol Vis Sci 44(3):1142-1149. https://doi.org/10.1167/iovs.02-0485

Ledee DR, Iovieno A, Miller D, Mandal N, Diaz M, Fell J, Fini ME, Alfonso EC (2009) Molecular identification of T4 and T5 genotypes in isolates from *Acanthamoeba* keratitis patients. J Clin Microbiol 47:1458–1462. https://doi.org/10.1128/JCM.02365-08

Li W, Wang Z, Qu J, Zhang Y, Sun X (2019) *Acanthamoeba* keratitis related to contact lens use in a tertiary hospital in China. BMC Ophthalmol. 19(1):202. https://doi.org/10.1186/s12886-019-1210-2

Lorenzo-Morales J, Khan NA, Walochnik J (2015) An update on *Acanthamoeba* keratitis: diagnosis, pathogenesis and treatment. Parasite 22:10. https://doi.org/10.1051/parasite/2015010

Lorenzo-Morales J, Martínez-Carretero E, Batista N, Álvarez-Marín J, Bahaya Y, Walochnik J, Valladares B (2007) Early diagnosis of amoebic keratitis due to a mixed infection with *Acanthamoeba* and *Hartmannella*. Parasitol Res 102:167–169. https://doi.org/10.1007/s00436-007-0754-x

Lorenzo-Morales J, Martín-Navarro CM, López-Arencibia A, Arnalich-Montiel F, Piñero JE, Valladares B (2013) *Acanthamoeba* keratitis: an emerging disease gathering importance worldwide? Trends in Parasitology 29:4. https://doi.org/10.1016/j.pt.2013.01.006

Lorenzo-Morales J, Morcillo-Laiz R, Martín-Navarro CM, López-Vélez R, López-Arencibia A, Arnalich-Montiel F, Maciver SK, Valladares, Martínez-Carretero E (2011) *Acanthamoeba* keratitis due to genotype T11 in a rigid gas permeable contact lens wearer in Spain. Cont Lens Anterior Eye 34(2):83-86. https://doi.org/10.1016/j.clae.2010.10.007

Maghsood AH, Sissons J, Rezaian M, Nolder D, Warhurst D, Khan NA (2005) *Acanthamoeba* genotype T4 from the UK and Iran and isolation of the T2 genotype from clinical isolates. J Med Microbiol 54:755–759. https://doi.org/10.1099/jmm.0.45970-0

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

Martín-Pérez T, Criado-Fornelio A, Martínez J, Blanco MA, Fuentes I, Pérez-Serrano J (2017) Isolation and molecular characterization of *Acanthamoeba* from patients with keratitis in Spain. Eur J Protistol 61:244–252. https://doi.org/10.1016/j.ejop.2017.06.009

Martín-Pérez T, Lozano-Cruz T, Criado-Fornelio A, Ortega P, Gómez R, La Mata FJ, Pérez-Serrano J (2019) Synthesis and *in vitro* activity of new biguanide-containing dendrimers on pathogenic isolates of *Acanthamoeba polyphaga* and *Acanthamoeba griffini*. Parasitol Res 118(6):1953-1961. https://doi.org/10.1007/s00436-019-06341-7

Maycock NJR, Jayaswal R (2016) Update on *Acanthamoeba* Keratitis. Cornea 35:713–20. https://doi.org/10.1097/ICO.0000000000000804

Megha K, Sharma M, Gupta A, Sehgal R, Khurana S (2018) Protein profiling of *Acanthamoeba* species using MALDI-TOF MS for specific identification of *Acanthamoeba* genotype. Parasitol Res 117(3):729-736. https://doi.org/doi:10.1007/s00436-017-5743-0

Mirjalali H, Niyyati M, Abedkhojasteh H, Babaei Z, Sharifdini M, Rezaeian M (2013) Pathogenic assays of *Acanthamoeba* belonging to the t4 genotype. Iran J Parasitol 8(4):530-535.

Moura H, Ospina M, Woolfitt AW, Barr JR, Visvesvara GS (2003a) Analysis of four human microsporidian isolates by MALDI-TOF mass spectrometry. J Eukaryot Microbiol 50: 156–163. https://doi.org/10.1111/j.1550-7408.2003.tb00110.x

Moura H, Woolffitt AR, Arrowood MJ, Visvesvara GS, Barr JR (2003b). Detection of protein fingerprints of emerging and opportunistic pathogens by MALDI-TOF MS and proteome database search. Amer J Mass Spectrometry 14: 615. https://doi.org/10.1111/jeu.12178

Mubareka S, Alfa M, Harding GK, Booton G, Ekins M, VanCaeseele P (2006) *Acanthamoeba* species keratitis in a soft contact lens wearer molecularly linked to well water. Can J Infect Dis Med Microbiol 17:2.

Munguía BC, Molina MO, Gonzáles-Lázaro M, Gonzáles Lezrobles AG, Bonilla P, Martínez-Palomo A (2005) Ultrastructural Study of Encystation and Excystation in *Acanthamoeba castellanii*. J Eukaryot Microbiol 52(2):153–158. https://doi.org/10.1111/j.1550-7408.2005.04-3273.x

Nagyová V, Nagy A, Janecek S, Timko J (2010a) Morphological, physiological, molecular and phylogenetic characterization of new environmental isolates of *Acanthamoeba* spp. from the region of Bratislava, Slovakia. Biologia 65:81–91. https://doi.org/10.2478/s11756-009-0217-1

Nagyová V, Nagy A, Timko J (2010b) Morphological, physiological and molecular biological characterisation of isolates from first cases of *Acanthamoeba* keratitis in Slovakia. Parasitol Res 106(4):861-872. https://doi.org/10.1007/s00436-010-1731-3

Nakagawa H, Hattori T, Koike N, Ehara T, Fujita K, Takahashi H, Kumakura S, Kuroda M, Matsumoto T, Goto H (2015) Investigation of the Role of Bacteria in the Development of *Acanthamoeba* Keratitis. Cornea 34(10):1308-1315. https://doi.org/10.1097/ICO.0000000000000541

Nakaminami H, Tanuma K, Enomoto K, Yoshimura Y, Onuki T, Nihonyanagi S, Hamada Y, Noguchi N (2017) Evaluation of In Vitro Antiamoebic Activity of Antimicrobial Agents Against Clinical *Acanthamoeba* Isolates. J Ocul Pharmacol Ther 33(8):629-634. https://doi.org/10.1089/jop.2017.0033

Nguyen TH, Weisenthal RW, Florakis GJ, Reidy JJ, Gaster RN, Tom D (2010) Penetrating

keratoplasty in active *Acanthamoeba* keratitis. Cornea 29:1000–1004. https://doi.org/10.1097/ICO.0b013e3181cc79a1

Niyyati M, Dodanghe S (2015) Enrichment of *Acanthamoeba* Culture Medium Using TYIS 33 Medium: a Steptoward a Successful Axenic Cultivation. Novel Biomed 3(2):69-72.

Niyyati M, Lorenzo-Morales J, Rezaie S, Rahimi F, Martín-Navarro CM, Mohebali M, Maghsood AH, Farnia S, Valladares B, Rezaeian M (2010) First report of a mixed infection due to *Acanthamoeba* genotype T3 and Vahlkampfia in a cosmetic soft contact lens wearer in Iran. Exp Parasitol 126(1):89-90. https://doi.org/10.1016/j.exppara.2009.10.009

Niyyati M, Lorenzo-Morales J, Rezaie S, Rahimi F, Mohebali M, Maghsood AH, Motevalli-Haghi A, Martín-Navarro CM, Farnia S, Valladares B, Rezaeian M (2009) Genotyping of *Acanthamoeba* isolates from clinical and environmental specimens in Iran. Exp Parasitol 121(3):242-245. https://doi.org/10.1016/j.exppara.2008.11.003

Niyyati M, Rezaeian M (2015) Current Status of *Acanthamoeba* in Iran: A Narrative Review Article. Iran J Parasitol 10(2):157-163.

Niyyati M, Sasani R, Mohebali M, Ghazi-Khansari M, Kargar F, Hajialilo E, Rezaeian M (2018) Anti-*Acanthamoeba* Effects of Silver and Gold Nanoparticles and Contact Lenses Disinfection Solutions. Iran J Parasitol 13(2):180-185.

Noorjahan NP (2010) Pathogenesis of *Acanthamoeba* keratitis. Ocul Surf 8:70–79. https://doi.org/10.1016/s1542-0124(12)70071-x

Nunes TET, Brazil NT, Fuentefria AM, Rott MB (2016) *Acanthamoeba* and *Fusarium* interactions: A possible problem in keratitis. Acta Tropica 157:102–107. https://doi.org/10.1016/j.actatropica.2016.02.001

Nuprasert W, Putaporntip C, Pariyakanok L, Jongwutiwes S (2010) Identification of a novel t17 genotype of *Acanthamoeba* from environmental isolates and t10 genotype causing keratitis in Thailand. J Clin Microbiol 48(12):4636-4640. https://doi.org/10.1128/JCM.01090-10

Oddo BD (2006) Infections caused by free-living amebas. Historical commentaries, taxonomy and nomenclature, protozoology and clinicopathologic features. Rev Chilena Infectol 23:200-14. http://dx.doi.org/10.4067/S0716-10182006000300002

Omaña-Molina M, González-Robles A, Salazar-Villatoro LI, Lorenzo-Morales J, Cristóbal-Ramos AR, Hernández-Ramírez VI, Talamás-Rohana P, Cruz ARM, Adolfo Martínez-Palomo A (2013) Reevaluating the Role of Acanthamoeba Proteases in Tissue Invasion: Observation of Cytopathogenic Mechanisms on MDCK Cell Monolayers and Hamster Corneal Cells. BioMed Research International 2013:13. http://dx.doi.org/10.1155/2013/461329

Omaña-Molina M, Vanzzini-Zago V, Hernandez-Martínez D, Gonzalez-Robles A, Salazar-Villatoro L, Ramirez-Flores E, Oregon-Miranda E, Lorenzo-Morales J, Martinez-Palomo A (2016) *Acanthamoeba* genotypes T3 and T4 as causative agents of amoebic keratitis in Mexico. Parasitol Res 115(2):873-878.

https://doi.org/10.1007/s00436-015-4821-4

Omaña-Molina M, Vanzzini-Zago V, Hernández-Martínez D, Reyes-Batlle M, Castelan-Ramírez I, Hernández-Olmos P, Salazar-Villatoro L, González-Robles A, Ramírez-Flores E, Servín-Flores C, Flores-Alvarado V, Alcántara-Castro M, Lorenzo-Morales J (2019) *Acanthamoeba* keratitis in Mexico: Report of a clinical case and importance of sensitivity assays for a better outcome. Exp Parasitol 196:22-27. https://doi.org/doi:10.1016/j.exppara.2018.11.005

Orosz E, Kriskó D, Shi L, Sándor GL, Kiss HJ, Seitz B, Nagy ZZ, Szentmáry N (2019) Clinical course of *Acanthamoeba* keratitis by genotypes T4 and T8 in Hungary. Acta Microbiol Immunol Hung 66(3):289-300. https://doi.org/10.1556/030.66.2019.008

Orosz E, Szentmáry, Kiss HJ, Farkas A, Kucsera I, Nagy ZZ (2018) First report of *Acanthamoeba* genotype T8 human keratitis. Acta Microbiologica et Immunologica Hungarica 65(1):73–79. https://doi.10.1556/030.65.2018.007

Ozkoc S, Tuncay S, Delibas SB, Akisu C, Ozbek Z, Durak I, Walochnik J (2008) Identification of *Acanthamoeba* genotype T4 and *Paravahlkampfia* sp. from two clinical samples. J Med Microbiol 57:392-396. https://doi.org/10.1099/jmm.0.47650-0

Padzik M, Baltaza W, Szaflik JP, Hendiger E, Dybicz M, Chomicz L (2018) Comparison of chlorhexidine disinfectant in vitro effect on environmental and ocular *Acanthamoeba* strains, the amoebic agents of human keratitis − an emerging sight-threatening corneal disease in Poland. Ann Parasitol 64(3):229-233. https://doi.org/10.17420/ap6403.157

Padzik M, Starościak B, Szaflik JP, Pietruczuk-Padzik A, Siczek P, Chomicz L (2016) Assessment of in vitro dynamics of pathogenic *Acanthamoeba* strains originating from contact lens wearers with infectious keratitis. Annals of Parasitology 62(4):331–336. https://doi.org/10.17420/ap6204.69

Padzik M, Szaflik JP, Baltaza W, Perkowski K, Dybicz M, Chomicz L (2017) In vivo confocal microscopy and *in vitro* culture techniques as tools for evaluation of severe *Acanthamoeba* keratitis incidents. Annals of Parasitology 63(4):341–346. https://doi.org/10.17420/ap6304.121

Page MA, Mathers WD (2013) *Acanthamoeba* keratitis: a 12-year experience covering a wide spectrum of presentations diagnoses and outcomes. J Ophthalmol 2013:670242. https://doi.org/10.1155/2013/670242

Patel A, Hammersmith K (2008) Contact lens-related microbial keratitis: recent outbreaks. Curr Opin Ophthalmol 19:302–306. https://doi.org/10.1097/ICU.0b013e3283045e74

Paterson GN, Rittig M, Siddiqui R, Khan NA (2011) Is *Acanthamoeba* pathogenicity associated with intracellular bacteria? Experimental Parasitology, 129:207-210. https://doi.org/10.1016/j.exppara.2011.06.017.

Possamai CO, Loss AC, Costa AO, Falqueto A, Furst C (2018) *Acanthamoeba* of three morphological groups and distinct genotypes exhibit variable and weakly inter-related physiological properties. Parasitol Res 117(5):1389-1400.

https://doi.org/10.1007/s00436-018-5824-8

Prithiviraj SR, Rajapandian SGK, Gnanam H, Gunasekaran R, Mariappan P, Singh SS, Prajna L (2020) Clinical presentations, genotypic diversity and phylogenetic analysis of *Acanthamoeba* species causing keratitis. J Med Microbiol 69(1):87-95. https://doi.org/10.1099/jmm.0.001121

Qvarnstrom Y, Visvesvara GS, Sriram R, Silva AJ (2006) Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. J Clin Microbiol 44:3589-95. https://doi.org/10.1128/JCM.00875-06

Rahman MM, Yagita K, Kobayashi A, Oikawa Y, Hussein AIA, Matsumura T, Tokoro M (2013) Genetic characterization of clinical *Acanthamoeba* isolates from Japan using nuclear and mitochondrial small subunit ribosomal RNA. Korean J Parasitol 51(4):401-411. https://doi.org/10.3347/kjp.2013.51.4.401

Ren M, Wu X (2010) Evaluation of three different methods to establish animal models of *Acanthamoeba* keratitis. Yonsei Medical Journal 51:121-127. https://doi.org/10.3349/ymj.2010.51.1.121

Risler A, Coupat-Goutaland B, Pelandakis M (2013) Genotyping and phylogenetic analysis of *Acanthamoeba* isolates associated with keratitis. Parasitol Res 112:3807–3816. https://doi.org/10.1007/s00436-013-3572-3

Rocha-Cabrera P, Reyes-Batlle M, Martín-Navarro CM, Dorta-Gorrín A, López-Arencibia A,

Sifaoui I, Martínez-Carretero E, Piñero JE, Martín-Barrera F, Valladares B, Lorenzo-Morales J (2015) Detection of *Acanthamoeba* on the ocular surface in a Spanish population using the Schirmer strip test: pathogenic potential, molecular classification and evaluation of the sensitivity to chlorhexidine and voriconazole of the isolated *Acanthamoeba* strains. J Med Microbiol 64(8):849-853. https://doi.org/10.1099/jmm.0.000103

Rodríguez-Martín J, Rocha-Cabrera P, Reyes-Batlle M, López-Arencibia A, Sifaoui I, Rizo-Liendo A, Bethencourt-Estrella CJ, Piñero JE, Lorenzo-Morales J (2018) Presence of *Acanthamoeba* in the ocular surface in a Spanish population of contact lens wearers. Acta Parasitol 63(2):393-396. https://doi.org/10.1515/ap-2018-0045

Sant'Ana VP, Foronda AS, Freitas D, Carrijo-Carvalho LC, Carvalho FRS (2017) Sensitivity of Enzymatic Toxins from Corneal Isolate of *Acanthamoeba* Protozoan to Physicochemical Parameters. Curr Microbiol 74:1316–1323. https://doi.org/10.1007/s00284-017-1319-6

Scheid PL, Balczun C (2017) Failure of molecular diagnostics of a keratitis-inducing *Acanthamoeba* strain. Exp Parasitol 183:236-239. https://doi.org/10.1016/j.exppara.2017.09.015

Schroeder JM, Booton GC, Hay J, Nisz IA, Seal DV, Markus MB, Fuerst PA, Byers TJ (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

Schuster FL, Visvesvara GS (2004) Free-living amoebae as opportunistic and

non-opportunistic pathogens of humans and animals. Int J Parasitol 34:1001-27. https://doi.org/10.1016/j.ijpara.2004.06.004

Sharifi N, Botero-Kleiven S, Ohman D, Barragan A, Winiecka-Krusnell J (2010) Genotypic characterization of *Acanthamoeba* spp. causing ocular infections in Swedish patients: identification of the T15 genotype in a case of protracted keratitis. Scand J Infect Dis 42:781–786. https://doi.org/10.3109/00365548.2010.490563

Sharma R, Jhanji V, Satpathy G, Sharma N, Khokhar S, Agarwal T (2013) Coinfection with *Acanthamoeba* and *Pseudomonas* in Contact Lens Y Associated Keratitis. Optometry and Vision Science, 90:2. https://doi.org/10.1097/OPX.0b013e31827f15b4

Sharma S, Pasricha G, Das D, Aggarwal RK (2004) *Acanthamoeba* keratitis in non-contact lens wearers in India: DNA typing-based validation and a simple detection assay. Arch Ophthalmol 122(10):1430-1434. https://doi.org/10.1001/archopht.122.10.1430

Shoff M, Joslin CE, Tu EY, Kubatko L, Fuerst PA (2008) Efficacy of contact lens systems against recent clinical and tap water *Acanthamoeba* isolates. Cornea 27: 713–719. https://doi.org/10.1097/QAI.0b013e31815e7251

Shoff M, Rogerson A, Schatz S, Seal D (2007) Variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions. Optom Vis Sci 84:202–207. https://doi.org/10.1097/OPX.0b013e3180339f81

Siddiqui R, Dudley R, Khan NA (2012) *Acanthamoeba* differentiation: a two-faced drama of

Dr Jekyll and Mr Hyde. Parasitology 139:826–834. https://doi.org/10.1017/S0031182012000042

Siddiqui R, Khan NA (2012) Biology and pathogenesis of *Acanthamoeba*. Parasites & Vectors 5:6. https://doi.org/10.1186/1756-3305-5-6.

Spanakos G, Tzanetou K, Miltsakakis D, Patsoula E, Malamou-Lada E, Vakalis NC (2006) Genotyping of pathogenic Acanthamoebae isolated from clinical samples in Greece-report of a clinical isolate presenting T5 genotype. Parasitol Int 55(2):147-149. https://doi.org/10.1016/j.parint.2005.12.001

Stothard DR, Schroeder-Diedrich JM, Awwad MH, Gast RJ, Ledee DR, Rodriguez-Zaragoza S, Dean CL, Fuerst PA, Byers TJ (1998) The evolutionary history of the genus *Acanthamoeba* and the identification of eight new 18S rRNA gene sequence types. J Eukaryot Microbiol 45:45–54. https://doi.org/10.1111/j.1550-7408.1998.tb05068.x

Taher EE, Méabed EMH, Abdallah I, Wahed WA (2018) *Acanthamoeba* keratitis in noncompliant soft contact lenses users: genotyping and risk factors, a study from Cairo, Egypt. Journal of Infection and Public Health 11: 377-383. https://doi.org/10.1016/j.jiph.2017.09.013

Takaoka-Sugihara N, Yamagami S, Yokoo S, Matsubara M, Yagita K (2012) Cytopathic effect of *Acanthamoeba* on human corneal fibroblasts. Mol Vis 18:2221-2228.

Tananuvat N, Techajongjintana N, Somboon P, Wannasan A (2019) The First *Acanthamoeba*

keratitis Case of Non-Contact Lens Wearer with HIV Infection in Thailand. Korean J Parasitol 57(5):505-511. https://doi.org/10.3347/kjp.2019.57.5.505

Tawfeek GM, Bishara SAH, Sarhan RM, Taher EE, Khayyal AE (2016) Genotyping, physiological and biochemical characterization of potentially pathogenic *Acanthamoeba* isolated from the environment in Cairo, Egypt. Parasitology Research 115: 1871-1881 https://doi.org/10.1007/s00436-016-4927-3

Visvesvara GS (2010) Free-living amebae as opportunistic agents of human disease. Journal of Neuroparasitology. https://doi.org/10.4303/jnp/N100802

Visvesvara GS, Moura H, Schuster FL (2007) Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol 50:1–26 https://doi.org/10.1111/j.1574-695X.2007.00232.x

Visvesvara GS, Schuster FL (2008) Opportunistic free-living amebae, part I. Clin Microbiol Newsl 30:151–158. https://doi.org/10.1016/j.clinmicnews.2008.09.004

Wagner C, Reyes-Batlle M, Ysea MA, Pérez MVG, Rondón CG, Paduani AJN, Pérez AD, López-Arencibia A, Sifaoui I, Galindo MVP, Suárez EP, Martínez-Carretero E, Valladares B, Piñero JE, Lorenzo-Morales J (2016) Genotyping of clinical isolates of *Acanthamoeba* genus in Venezuela. Acta Parasitol 61(4):796-801. https://doi.org/10.1515/ap-2016-0110

Walochnik J, Scheikl U, Haller-Schober EM (2015) Twenty years of *Acanthamoeba*

diagnostics in Austria. Journal of Eukaryotic Microbiology 62: 3-11. https://doi.org/10.1111/jeu.12149

Wesolowska M, Cisowska A, Myjak P, Marek J, Jurowskaka-Liput J, Jakubaszko J (2006) *Acanthamoeba* keratitis in contact lens wearers in Poland. Advances in Clinical and Experimental Medicine 15:553-555.

Wilhelmus KR, Jones DB, Matoba AY, Hamill MB, Pfugfelder SC, Weikert MP (2008) Bilateral *Acanthamoeba* keratitis. American Journal of Ophthalmology 145:193-197. https://doi.org/10.1016/j.ajo.2007.09.037

Xuan YH, Yu HS, Jeong HJ, Seol SY, Chung DI, Kong HH (2007) Molecular characterization of bacterial endosymbionts of *Acanthamoeba* isolates from infected corneas of Korean patients. Korean J Parasitol 45(1):1-9. https://doi.org/10.3347/kjp.2007.45.1.1

Yera H, Zamfir O, Bourcier T, Ancelle T, Batellier L, Dupouy-Camet J, Chaumeil C (2007) Comparison of PCR, microscopic examination and culture for the early diagnosis and characterization of *Acanthamoeba* isolates from ocular infections. Eur J Clin Microbiol Infect Dis 26(3):221-224. https://doi.org/10.1007/s10096-007-0268-6

Yera H, Zamfir O, Bourcier T, Viscogliosi E, Noël C, Dupouy-Camet J, Chaumeil C (2008) The genotypic characterisation of *Acanthamoeba* isolates from human ocular samples. Br J Ophthalmol 92(8):1139-1141. https://doi.org/10.1136/bjo.2007.132266

Zhang Y, Sun X, Wang Z, Li R, Luo S, Jin X, Deng S, Chen W (2004) Identification of 18S ribosomal DNA genotype of *Acanthamoeba* from patients with keratitis in North China. Invest Ophthalmol Vis Sci 45(6):1904-1907. https://doi.org/10.1167/iovs.03-1073

Zhao G, Sun S, Zhao J, Xie L (2010) Genotyping of *Acanthamoeba* isolates and clinical characteristics of patients with Acanthamoeba keratitis in China. J Med Microbiol 59: 462–466. https://doi.org/10.1099/jmm.0.016667-0

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Contents

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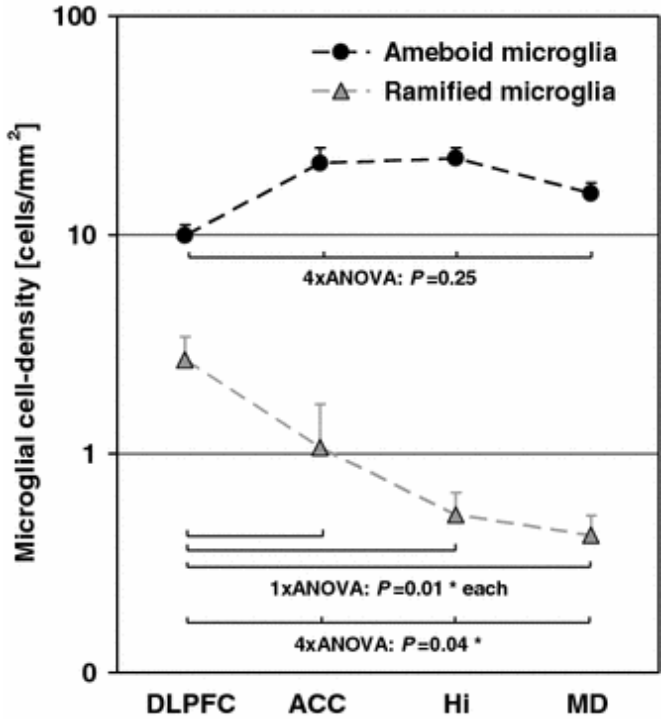

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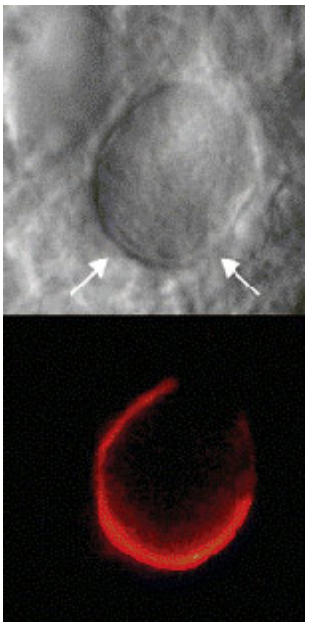

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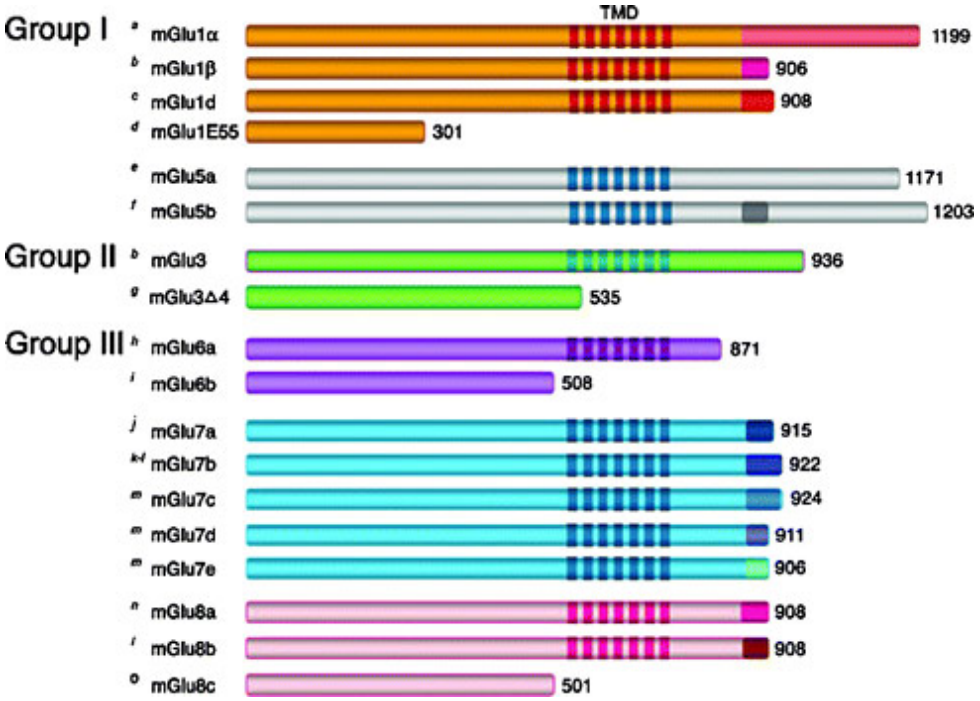

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- The author's institution may be informed
- A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.

Fundamental errors

Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

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Authorship principles

These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, **before** the work is submitted.

The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

2) drafted the work or revised it critically for important intellectual content;

3) approved the version to be published; and

4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

* Based on/adapted from:

ICMJE, Defining the Role of Authors and Contributors,

Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

Disclosures and declarations

All authors are requested to include information regarding sources of funding, financial or non-financial interests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).

The decision whether such information should be included is not only dependent on the scope of the journal, but also the scope of the article. Work submitted for publication may have implications for public health or general welfare and in those cases it is the responsibility of all authors to include the appropriate disclosures and declarations.

Data transparency

All authors are requested to make sure that all data and materials as well as software application or custom code support their published claims and comply with field standards. Please note that journals may have individual policies on (sharing) research data in concordance with disciplinary norms and expectations.

Role of the Corresponding Author

One author is assigned as Corresponding Author and acts on behalf of all co-authors and ensures that questions related to the accuracy or integrity of any part of the work are appropriately addressed.

The Corresponding Author is responsible for the following requirements:

- ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors;
- managing all communication between the Journal and all co-authors, before and after publication;*
- providing transparency on re-use of material and mention any unpublished material (for example manuscripts in press) included in the manuscript in a cover letter to the Editor;
- making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate (see above).

* The requirement of managing all communication between the journal and all co-authors during submission and proofing may be delegated to a Contact or Submitting Author. In this case please make sure the Corresponding Author is clearly indicated in the manuscript.

Author contributions

In absence of specific instructions and in research fields where it is possible to describe discrete efforts, the Publisher recommends authors to include contribution statements in the work that specifies the contribution of every author in order to promote transparency. These contributions should be listed at the separate title page.

Examples of such statement(s) are shown below:

• Free text:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name], [full name] and [full name]. The first draft of the manuscript was written by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Example: CRediT taxonomy:

• Conceptualization: [full name], …; Methodology: [full name], …; Formal analysis and investigation: [full name], …; Writing - original draft preparation: [full name, …]; Writing - review and editing: [full name], …; Funding acquisition: [full name], …; Resources: [full name], …; Supervision: [full name],….

For **review articles** where discrete statements are less applicable a statement should be included who had the idea for the article, who performed the literature search and data analysis, and who drafted and/or critically revised the work.

For articles that are based primarily on the **student's dissertation or thesis**, it is recommended that the student is usually listed as principal author:

A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

Affiliation

The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may additionally be stated. Addresses will not be updated or changed after publication of the article.

Changes to authorship

Authors are strongly advised to ensure the correct author group, the Corresponding Author, and the order of authors at submission. Changes of authorship by adding or deleting authors, and/or changes in Corresponding Author, and/or changes in the sequence of authors are **not** accepted **after acceptance** of a manuscript.

- **Please note that author names will be published exactly as they appear on the accepted submission!**

Please make sure that the names of all authors are present and correctly spelled, and that addresses and affiliations are current.

Adding and/or deleting authors at revision stage are generally not permitted, but in some cases it may be warranted. Reasons for these changes in authorship should be explained. Approval of the change during revision is at the discretion of the Editor-in-Chief. Please note that journals may have individual policies on adding and/or deleting authors during revision stage.

Author identification

Authors are recommended to use their ORCID ID when submitting an article for consideration or acquire an ORCID ID via the submission process.

Deceased or incapacitated authors

For cases in which a co-author dies or is incapacitated during the writing, submission, or peer-review process, and the co-authors feel it is appropriate to include the author, co-authors should obtain approval from a (legal) representative which could be a direct relative.

Authorship issues or disputes

In the case of an authorship dispute during peer review or after acceptance and publication, the Journal will not be in a position to investigate or adjudicate. Authors will be asked to resolve the dispute themselves. If they are unable the Journal reserves the right to withdraw a manuscript from the editorial process or in case of a published paper raise the issue with the authors' institution(s) and abide by its guidelines.

Confidentiality

Authors should treat all communication with the Journal as confidential which includes correspondence with direct representatives from the Journal such as Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit consent has been received to share information.

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Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

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Conflicts of Interest / Competing Interests

Authors are requested to disclose interests *that are directly or indirectly related to the work submitted for publication*. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

Interests that should be considered and disclosed but are not limited to the following:

Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.

Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).

Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.

Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a subject. Review articles therefore require a disclosure statement.Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

Please note that, in addition to the above requirements, funding information (given that funding is a potential conflict of interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is **not added** to the manuscript itself. Under ‘summary of requirements’ (see below) funding information should be included in the ‘**Declarations**’ section.

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Funding’ and/or ‘Conflicts of interests’/’Competing interests’. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors’ contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

- Partial financial support was received from [...]
- The research leading to these results received funding from […] under Grant Agreement No[…].
- This study was funded by […]
- This work was supported by […] (Grant numbers […] and […]

Examples of statements to be used when there is no funding:

- The authors did not receive support from any organization for the submitted work.
- No funding was received to assist with the preparation of this manuscript.
- No funding was received for conducting this study.
- No funds, grants, or other support was received.

Examples of statements to be used when there are interests to declare:

- **Financial interests:** Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.

  Non-financial interests: Author C is an unpaid member of committee Z.

- **Financial interests:** The authors declare they have no financial interests.

  Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

- **Financial interests:** Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.

  Non-financial interests: none.

- **Financial interests:** Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.

  Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no conflicts of interest to declare that are relevant to the content of this article.
- All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

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Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in

accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: *Filip1*$^{tm1a(KOMP)Wtsi}$ **RRID:MMRRC_055641-UCD**

Cell Line: RST307 cell line **RRID:CVCL_C321**

Antibody: Luciferase antibody DSHB Cat# LUC-3, **RRID:AB_2722109**

Plasmid: mRuby3 plasmid **RRID:Addgene_104005**

Software: ImageJ Version 1.2.4 **RRID:SCR_003070**

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as “A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered’ should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal’s Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

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Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles

unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

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Research involving animals, their data or biological material

The welfare of animals (vertebrate and higher invertebrate) used for research, education and testing must be respected. Authors should supply detailed information on the ethical treatment of their animals in their submission. For that purpose they may use the ARRIVE checklist which is designed to be used when submitting manuscripts describing animal research.

For studies involving client-owned animals, authors must also document informed consent from the client or owner and adherence to a high standard (best practice) of veterinary care.

Authors are recommended to comply with:

• The International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and consult the IUCN red list index of threatened species.

• Convention on the Trade in Endangered Species of Wild Fauna and Flora

When reporting results authors should indicate:

• … that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted. Please provide the name of ethics committee and relevant permit number;

• … whether the legal requirements or guidelines in the country and/or state or province for the care and use of animals have been followed.

Researchers from countries without any legal requirements or guidelines voluntarily should refer to the following sites for guidance:

– The Basel Declaration describes fundamental principles of using animals in biomedical research

– The International Council for Laboratory Animal Science (ICLAS) provides ethical guidelines for researchers as well as editors and reviewers

– The Association for the study of Animal Behaviour describes ethical guidelines for the treatment of animals in research and teaching

– The International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics provide guidelines for authors on animal ethics and welfare

Researchers may wish to consult the most recent (ethical) guidelines available from relevant taxon-oriented professional societies.

If a study was granted exemption or did not require ethics approval, this should also be detailed in the manuscript.

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (No. 29 12 2014, Kocaeli, Turkey).

• All procedures performed in the study were in accordance with the ARVO Statement for Use of Animals in Ophthalmic Vision and Research. The ethical principles established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 2011) were followed. The research protocol was approved by the Ethics Committee on Animal Use (Protocol No. 06174/14) of FCAV/Unesp, Jaboticabal.

• This study involved a questionnaire-based survey of farmers as well as blood sampling from their animals. The study protocol was assessed and approved by Haramaya University, research and extension office. Participants provided their verbal informed consent for animal blood sampling as well as for the related survey questions. Collection of blood samples was carried out by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare.

• All brown bear captures and handling were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Application C18/15) and the Swedish Environmental Protection Agency in compliance with Swedish laws and regulations.

• The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the University of Maiduguri Senate committee on Medical Research ethics. Proper permit and consent were obtained from the Maiduguri abattoir management, before the faecal samples of the cattle and camels slaughtered in this abattoir were used for this experiment.

Examples of statements to be used when no ethical approval is required/exemption granted:

• No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

• As the trappings of small mammals were conducted as part of regular pest control measures in accordance with the NATO Standardized Agreement 2048 "Deployment Pest and Vector Surveillance and Control ", no approval by an ethics committee was required.

• All experiments have been conducted as per the guidelines of the Institutional Animal Ethics Committee, Department of Zoology, Utkal University, Bhubaneswar, Odisha, India. However, the insect species used in this study is reared for commercial production of raw silk materials, as a part of agro-based industry. Therefore, use of this animal in research does not require ethical clearance. We have obtained permission from the office of Research officer sericulture, Baripada, Orissa, India for the provision of infrastructure and support for rearing of silkworm both in indoor and outdoor conditions related to our study to promote sericulture practices.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

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Research Data Policy

A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

The journal strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature’s information on recommended repositories.

List of Repositories

Research Data Policy

General repositories - for all types of research data - such as figshare and Dryad may be used where appropriate.

Datasets that are assigned digital object identifiers (DOIs) by a data repository may be cited in the reference list. Data citations should include the minimum information recommended by DataCite: authors, title, publisher (repository name), identifier.

DataCite

Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. Persistent identifiers (such as DOIs and accession numbers) for relevant datasets must be provided in the paper

For the following types of data set, submission to a community-endorsed, public repository is mandatory:

Mandatory deposition	Suitable repositories
Protein sequences	Uniprot
	Genbank
DNA and RNA sequences	DNA DataBank of Japan (DDBJ)
	EMBL Nucleotide Sequence Database (ENA)
DNA and RNA sequencing data	NCBI Trace Archive

Genetic polymorphisms	NCBI Sequence Read Archive (SRA) dbSNP dbVar European Variation Archive (EVA)
Linked genotype and phenotype data	dbGAP The European Genome-phenome Archive (EGA)
Macromolecular structure	Worldwide Protein Data Bank (wwPDB) Biological Magnetic Resonance Data Bank (BMRB) Electron Microscopy Data Bank (EMDB)
Microarray data (must be MIAME compliant)	Gene Expression Omnibus (GEO) ArrayExpress
Crystallographic data for small molecules	Cambridge Structural Database

For more information:

Research Data Policy Frequently Asked Questions

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