The new Chikungunya virus outbreak in Italy possibly originated from a single introduction from Asia

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Introduction

Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus belonging to the *Togaviridae* family [1]. Chikungunya is a Makonde word (*Bantu* language) meaning *The one which bends up*. The term is related to the posture that the patient acquires as a consequence of the excruciating joint pain [2]. The virus is the cause of Chikungunya fever (CHIKV fever), a febrile illness associated with severe arthralgia and rash [3–5]. CHIKV is transmitted by culicine mosquitoes and can affect both arthropods and vertebrates [6,7]. The arthropod vectors are infected throughout their life cycle and those most commonly involved in transmission to vertebrates are *Aedes aegypti*, *Aedes albopictus* and *Aedes polynesiensis*, although transmission mediated by *Culex* has also been rarely reported [1,8,9].

CHIKV probably originated in Africa [1,7,10], where it is thought to persist through a ‘sylvatic cycle’ involving wild primates and forest dwelling mosquitoes [6,7,9,11].

Upon CHIKV’s subsequent introduction in Asia, mainly carried by *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*, the virus began to spread more frequently in human populations through an urban transmission cycle [10,11].

During the epidemic in La Reunion, in 2005–2006 CHIKV underwent a specific mutation in the envelope (E) gene (Ala226Val) that allowed a better adaptation to *Aedes albopictus*, the only competent vector present on the island [1]. Viral strains with the same mutation were also identified in India and caused an outbreak in North-Eastern Italy [12,13]. In August–September 2017, a new outbreak has been reported in Anzio, a residential beach village 60 km from the south of Rome in Italy. As of 4 October, 239 cases in the Lazio region, were diagnosed. CHIKV strains isolated from one patient and one

mosquito were further sequenced to compare with sequences from previous epidemics [14].

In this study, the origin of CHIKV causing this recent outbreak was investigated by Bayesian phylogeographic analysis.

Material and methods

A data set of 110 CHIKV reference sequences of the E1 gene region, downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>), including the two recent Italian sequences isolated in the first two weeks of September 2017 in Anzio, was assembled and analyzed by Bayesian phylogeographic analysis. CHIKV evolutionary rate was estimated using the Markov Chain Monte Carlo (MCMC) approach implemented in BEAST v 1.8.2, under HKY+G model, enforcing either a strict or an uncorrelated long-normal relaxed clock model and different demographic priors. The MCMC chains were run for at least 200 million generations, and sampled every 20,000 steps. The run was halted when the effective sampling size of all model parameters were >200. The constant demographic model with a relaxed molecular clock best fit the data according to the Bayes factors (lnBF > 10 in all the comparisons).

Results and discussion

Phylogeographic analysis illustrated the spatial diffusion of CHIKV during the recent Italian epidemic. Despite non-uniform sampling of available sequences from NCBI based on geographical locations, that includes also under-representation of Italian sequences, our result pointed to a clear relationship between the recently isolated Italian CHIKV sequences and India and Pakistan

strains isolated in 2016 (posterior probability > 90%). The 2017 Italian strains clustered within a highly supported ($p > 0.9$) monophyletic clade (Figure 1). The Italian clade clustered, in turn, with a Pakistani sequence branching off a well-supported ($p > 0.9$) Indian clade part of the large CHIKV ECSA group that includes sequences from East, Central and South Africa. The most recent common ancestor (TMRCA) of the Italian clade dated from May 2017 (95% HPD: 1.67×10^{-4} – 4.20×10^{-4} 95%), as in Figure 1, a few months earlier than the first reported cases in August–September 2017 in Anzio.

The phylogeographic tree (Figure 1) clearly shows that the Italian sequences, isolated in September 2017 during the epidemic period in Anzio, have probably been introduced to Italy in May 2017 (posterior probability > 90%) from Pakistan where the epidemic is still ongoing [14].

Globalization has dramatically changed the way in which pathogens spread. Today, travel and population movement are the principal factors for which microorganisms can be introduced in new ecosystems originally unaffected. Travelers and population migration are unfortunately vehicles giving rise to unexpected epidemics.

The outbreak reported in Emilia-Romagna Region in the summer of 2007 represented the first epidemic reported in a temperate area, probably related to the summer season and the high concentration of *Aedes albopictus* in the area [15].

Since the first introduction of CHIKV in Europe, from Asia ten years ago, the recent Italian outbreak described in Anzio [14] highlights once more Asia as the origin, as

showed by the phylogeographic tree (Figure 1) where the recent Italian strain appears to be introduced from Pakistan. These data highlight as the introduction may occur from any endemic region experiencing frequent connection with Europe.

Bayesian and phylogeographic analyses have demonstrated, once more, to be powerful and efficient tools to discover the origin and the evolution of microorganisms potentially causing epidemics.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure 1. Maximum Clade credibility tree with Bayesian Phylogeography reconstruction inferred from CHIKV E1 sequences.

Notes: Branch lengths are scaled in time and colored by the inferred country of origin (internal branches) and geographic location of the sampled sequence (tip branches), according to the legend to the left. An asterisk along a branch represents robust statistical support (posterior probability > 0.9). Diamond represents the zoom of the Asian/Italian clade including the two new Italian isolates as well as the Pakistani and Indian sequences.

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