Cholera is still an important public health problem in several countries, including Thailand. In this study, a collection of clinical and environmental V. cholerae serogroup O1, O139, and non-O1/non-O139 strains originating from Thailand (1983 to 2013) was characterized to determine phenotypic and genotypic traits and to investigate the genetic relatedness. Using a combination of conventional methods and whole genome sequencing (WGS), 78 V. cholerae strains were identified. WGS was used to determine the serogroup, biotype, virulence, mobile genetic elements, and antimicrobial resistance genes using online bioinformatics tools. In addition, phenotypic antimicrobial resistance was determined by the minimal inhibitory concentration (MIC) test. The 78 V. cholerae strains belonged to the following serogroups O1: (n = 44), O139 (n = 16) and non-O1/non-O139 (n = 18). Interestingly, we found that the typical El Tor O1 strains were the major cause of clinical cholera during 1983-2000 with two Classical O1 strains detected in 2000. In 2004-2010, the El Tor variant strains revealed genotypes of the Classical biotype possessing either only ctxB or both ctxB and rstR while they harbored tcpA of the El Tor biotype. Thirty O1 and eleven O139 clinical strains carried CTXφ (Cholera toxin) and tcpA as well four different pathogenic islands (PAIs). Beside non-O1/non-O139, the O1 environmental strains also presented chxA and Type Three Secretion System (TTSS). The in silico MultiLocus Sequence Typing (MLST) discriminated the O1 and O139 clinical strains from other serogroups and environmental strains. ST69 was dominant in the clinical strains belonging to the 7th pandemic clone. Non-O1/non-O139 and environmental strains showed various novel STs indicating genetic variation. Multidrug-resistant (MDR) strains were observed and conferred resistance to ampicillin, azithromycin, nalidixic acid, sulfamethoxazole, tetracycline, and trimethoprim and harboured variants of the SXT elements. For the first time since 1986, the presence of V. cholerae O1 Classical was reported causing cholera outbreaks in Thailand. In addition, we found that V. cholerae O1 El Tor variant and O139 were pre-dominating the pathogenic strains in Thailand. Using WGS and bioinformatic tools to analyze both historical and contemporary V. cholerae circulating in Thailand provided a more detailed understanding of the V. cholerae epidemiology, which ultimately could be applied for control measures and management of cholera in Thailand.