Abstract: Plutarch, Arrian, Diodorus, Justin, and other ancient historians report that rumors of poisoning arose after the death of Alexander in Babylon in 323 BC. Alexander's close friends suspected a legendary poison gathered from the River Styx in Arcadia, so corrosive that only the hoof of a horse could contain it. It's impossible to know the real cause of Alexander's death, but a recent toxicological discovery may help explain why some ancient observers believed that Alexander was murdered with Styx poison. We propose that the river harbored a killer bacterium that can occur on limestone rock deposits. This paper elaborates on our Poster presentation, Toxicological History Room, XII International Congress of Toxicology, Barcelona, 19–23 July 2010, and Society of Toxicology Annual Meeting, Washington DC, March 2011.
THE DEADLY STYX RIVER
and the DEATH OF ALEXANDER THE GREAT

Adrienne Mayor and Antoinette Hayes

According to several ancient historians, rumors of poisoning circulated after the death of Alexander, at age 32, in Babylon in 323 BC (Bosworth 1971 and 2010; Lane Fox 2004). Some close friends suspected a legendary poison gathered from the Styx waterfall near Nonacris in Arcadia (north central Peloponnese, Greece), a substance reputed to be so corrosive it could only be contained in the hoof of a horse. Many ancient and modern writers have speculated on the true cause of Alexander’s death, which remains an unsolved mystery.

Ancient historians were divided on whether Alexander died of natural causes or was murdered by poison. Justin (12.13–14) and Pliny (Natural History 30.53) accepted the poison conspiracy (as did Pseudo–Plutarch Lives of the Ten Orators 56; Moralia 849 F); whereas Pausanias (8.17.18), Arrian (7.27), and Curtius (10.10.14) were neutral and Plutarch (Alexander 75–77) was skeptical. Diodorus 17.117.5–118.1–2 was cautious, pointing out that after Alexander’s bitter enemies, Antipater and Cassander, took over Alexander’s empire, “many historians did not dare to write about the drug” or the plot. Moreover, Alexander’s mother Olympias sought revenge on Cassander and others because she believed that they had poisoned her son (19.11.8; Olympias was murdered by Cassander). Diodorus’s intimations were more strongly stated by Justin (12.13), who wrote: “The conspiracy . . . was suppressed by the power of Alexander’s successors.” In the Middle Ages and Renaissance, poisoning was generally accepted as the cause of Alexander’s death (Barb 1971, 334n4). Voltaire, among others, accepted the poisoning plot (Smith 1922, 23). For a valuable summary and full bibliography of Alexander’s last days, with symptoms, timeline based on the ancient sources, and appendix of all the proposed causes of death and their merits and drawbacks, see Atkinson, Truter and Truter 2009.

Cases have been made for each possibility by numerous modern historians. This article does not focus on the controversy over whether or not a poison plot existed or was carried out. Instead, we investigate the
ancient beliefs surrounding the Styx’s poisonous water. Then we consider
the ancient literary evidence for the rumor that Alexander died from Styx
poison in light of modern geology and toxicology. We seek to answer the
following questions: Could the Styx watercourse have contained toxins? If
so, what was their nature? Why did Alexander’s friends suspect this
particular poison? Was it plausible for them to believe that a substance
from the Styx was capable of causing Alexander’s death?

We review the literature on the Styx poison and its alleged role in
Alexander’s demise. We conclude by proposing three hypotheses, which
if proven would identify the mysterious poisonous substance from the
Styx, feared since the time of Homer and Hesiod and believed by many to
have killed Alexander.¹

The Styx, River of Hades, in Greek Myth

Greek myth held that on the dark waters of the gloomy River Styx
(“Abhorrent”), portal to the Underworld, the gods swore their sacred
oaths (FIG 1). If they broke their word, Zeus forced them to drink a
golden cup of cold water taken from the “towering rocks” near Nonacris.
There the stream sometimes trickles and other times “plunges over
jagged mountains and cascades over a high, steep cliff.” The water
“strikes the gods down with an evil trance [coma]. Unable to move,
breathe, or eat, the sickened gods lose their voices for a year [and] are
cut off from the other gods for nine years” (Hesiod Theogony 775–819).²

In antiquity, the water of the Styx (Mavroneri River, see below) was said to
be deadly to mortals. The stream—the “original acid test”—may have
been the site of ancient poison ordeals (Frazer 1913, 253–54; quote,
Smith 1922, 22). The ancient Greek historian Herodotus (6.74.) related
that Arcadians swore loyalty oaths on the water of the Styx, “at the pool
ringed with stones” at the base of the waterfall over the high precipice,
near Nonacris and Pheneus. The geographer Strabo confirmed the
location: "Near Pheneus [in Arcadia] is the water of the Styx—a small
stream of deadly water held to be sacred." He remarked that Styx water
mixed with sulphur killed animals and plants (8.8.4; 14.2.7). Pliny (2.231)
included “the Styx near Nonacris” in his list of poisonous waters:
“Although the river is not peculiar in color or smell, drinking it causes
immediate death.”

In the second century AD, Pausanias (8.17–18) traveled west from
Pheneus, turning north over the Zaroukhla pass in the Chelmos (Aroania)
Mountains to the ruins of ancient Nonacris. “As you go from Pheneus to
the west, the path on the left leads to Cleitor, while on the right is the
road to Nonacris and the water of the Styx. . . . Not far from the ruins of
Nonacris is a high cliff; I know of none other that rises to so great a height.” Pausanias viewed the waterfall in summer: the Styx “trickles down the sheer rock face of the high crag, down through a rocky glen into the River Krathis, and its water is death to humans and animals.”

Early modern travelers in Greece made the arduous, dangerous ascent on foot to the site. For example, Col. William Leake visited the Styx in 1806, and reported that the locals called it Mavro Nero (“Black Water”) or “Dragon Water.” Alexander von Humboldt (1860, 194) commented that the stream had an “evil reputation” among “the present inhabitants.” In 1895, Sir James George Frazer retraced Pausanias’s journey to the waterfall and wrote a long, detailed description of the “awesome” landscape of massive, rugged peaks, and the local lore (1913, 248–55).

In the twentieth century, local people avoided drinking from the Styx/Mavroneri (“Black Water”) and reported that the water corrupts metal and clay vessels (Chisholm 1911). According to another modern traveler, “The present day inhabitants of the neighborhood speak of the stream as The Black Waters and The Terrible Waters, and still attribute to them some of the uncanny properties with which they were endowed by the ancients” (Smith 1922, 24).

Based on the ancient details of the impressive waterfall of located near Nonacris and Pheneus, in the northern slopes of the Chelmos range (2,355 meters, nearly 8,000 feet), west of Lake Stymphalos, the Styx (Υδάτα τῆς Στυγός) is securely identified as the Mavro Nero or Mavroneri (Μαυρονέρι, “Black Water”). The Mavroneri is a tributary of the Krathis (Κράθις) River (emptying into the Corinthian Gulf), now in the prefecture of Achaia (MAP, FIG 2). The stream flows north from its sources (at about 2,100 meters, nearly 7,000 feet), through dense forest and thick vegetation, cascading over a perpendicular limestone cliff more than 200 meters (over 650 feet) high, near the summit of Mt Chelmos, into a narrow, steep valley. This is the second highest waterfall in Greece. Today the falls can be viewed from a distance; the arduous footpath to the base of the falls follows part of an ancient road (wheelruts visible) from Feneos (Pheneus) or Kalavrita (Barber 1995, 330; see video “Mavroneri Waterfall” 2010).

Ancient authors described the water’s deleterious effect on vessels of various materials. According to a lost work of the fourth century BC by Theophrastus (cited by Pliny 2.213 and 2.231), the water of the Styx was poisonous (and so were its fish). The water corrupted all containers including keratina (horns and hooves), but it could be collected with a sponge fastened to a stick (Frazer 1913, 255). Several other ancient writers claim that only the hoof of a horse, mule, or ass could withstand the poison: for example, Pliny 30.149; Plutarch Alexander 77.4; Pseudo–
According to Pausanias (8.17–19), the “lethal power” of the Styx, “seemingly invented for the destruction of human beings,” was first recognized “once upon a time” after goats tasted the water and died. It “dissolves glass, crystal, murrhine, and pottery and corrupts horn and bone, iron, bronze, lead, tin, silver, and silver and gold alloy.” Pausanias continues, “The only thing able to resist corrosion is the hoof of a mule or horse. I have no actual knowledge that this was the poison that killed Alexander, but I have certainly heard it said.”

Aelian (ca AD 200, On Animals 10.40) also linked Alexander and the Styx, but without referring to the poison plot. “In Scythia, there are asses with horns, and these horns hold water from the river of Arcadia known as the Styx; all other vessels the water eats through, even iron. Now one of these horns, they say, was brought by Sopatros [the poet of Paphos?] to Alexander” who dedicated the horn to Apollo at Delphi “with this inscription: ‘In honor of the god of healing, Alexander of Macedon set up this horn from a Scythian ass, a marvelous object, which . . . withstood the strength of water from the Styx.’”

The relationship of Aelian’s story to the poisoning plot is unknown, but this is the first indication that at some point in later antiquity, the notion of a horn that resists poison was transformed into a belief that drinking cups of horn could neutralize poison. For example, in the third century AD, Philostratus (Apollonius of Tyana 3.2) reported that in India drinking cups made of rhinoceros horn ensure immunity against disease and poison. By the Middle Ages in Europe, unicorn horns and elk hooves were thought to repel venoms and poisons (see further discussion below).

**Alexander’s Death in Babylon, 323 BC**

During one of many all-night drinking parties in Babylon (now modern Iraq) in May 323 BC, Alexander’s companions heard him cry out in pain from a “sudden, stabbing agony in the liver.” The conqueror of the known world was put to bed with severe abdominal pains and very high fever. Over the next 12 days, his condition declined and he became gravely ill. Alexander suffered restlessness, weakness, extreme thirst, loss of consciousness, possible convulsions, and great pain. Partial paralysis set in—he was able to move his eyes, head, and fingers with difficulty. He lost the ability to speak, and fell into a deathlike state. (FIG 3)

Several ancient historians described his last days, citing contemporary texts that no longer survive, including a mysterious source called the “Royal
According to Diodorus (17.117, cf Arrian 7.27.2), at the last banquet he attended, Alexander “drank much unmixed [strong] wine . . . and finally gulped down a huge beaker. Instantly he shrieked aloud as if struck by a violent blow.” His attendants “conducted him to bed, and his physicians were summoned” but Alexander continued to suffer in great pain. Justin (12.13–15) adds details: “Taking up a cup, he had drunk half of it when he suddenly uttered a groan, as if he had been pierced by a spear; he was carried half-conscious from the banquet. The torture was so excruciating that he called for a sword to put an end to it. The pain upon being touched by his attendants was as if he were covered with wounds. . . On the sixth day, he could no longer speak.”

The historian Arrian (7.25–26) affirmed that Alexander was unable to speak: “Lying speechless as his men filed by, he struggled to raise his head, and in his eyes there was a look of recognition for each individual as he passed.” Justin (12.17) also noted that Alexander was speechless, but could move his hands: “Unable to speak, he took his ring from his finger, and gave it to Perdiccas.”

Plutarch (Alexander 75–77) denied the stabbing pain, describing only “a raging fever” marked by “violent thirst—he drank a lot of wine, upon which he fell into delirium.” Plutarch also described restlessness, loss of appetite, high fever, and inability to speak.

According to the third century AD Greek Alexander Romance 3.30–31 (Stoneman 1991), Alexander was killed with a poison that destroyed bronze, glass, and clay, and had to be sealed in a lead jar inside an iron jar. After his cupbearer Iolaus (or Iollus) served a beaker of wine late in the evening, Alexander began showing signs of malaise, getting up and pacing around the room. “He again sat down [and] with his hands trembling, complained that it was as if a heavy yoke were upon his neck. When he stood again to drink . . . he shouted with pain as if pierced in the liver with an arrow.” When he tried to induce vomiting to rid himself of the poison, Iolaus gave him a poisoned feather. “Racked with pain,” Alexander’s condition deteriorated, and he “could not speak because his tongue was so swollen.” He suffered convulsions, delirium/hallucinations, and bouts of unconsciousness: “Throughout the night the king would writhe and shake upon his bed, then he would become still. At other times he would ramble with meaningless words, appearing to speak with spirits in the bedchamber.” (FIG 4)

Thirteen days after falling ill at the banquet, Alexander was pronounced dead, on the afternoon of June 11, 323 BC, just before his 33rd birthday.
It was noted that his body was strangely preserved, even in Babylon’s hot climate, for several days after he was placed in a coffin in a storeroom (Plutarch *Alexander* 77; Curtius 9.19). This effect could indicate that some poison preserved the corpse, or that profound coma was mistaken for death.

Various modern theories for a natural cause of Alexander’s slow death have been proposed, including alcohol poisoning from heavy drinking, septicemia from infected old wounds, pancreatitis, malaria, typhoid, West Nile fever, or other unknown disease, accidentally harmful treatment by physicians (Atkinson, Truter, and Truter 2009), or a combination of causes. Before falling ill, Alexander had traveled in India and he was lost for three days in the Great Swamp of Babylonia (the marshy Tigris–Euphrates delta; Diodorus 17.116.5–7). Typhoid (Oldach et al 1998; Cunha 2004) and malaria (Engels 1978; first proposed by a French physician in 1878; Borza 2010) have been suggested, but it seems likely that these diseases would have struck others in Babylon with similar symptoms. Marr and Calisher (2003) suggested West Nile fever, but that is a recently evolved disease. According to the detailed historical accounts, Alexander was the only one to fall ill, which could point to accidental or deliberate poisoning.

Many modern arguments have been made for accidental poisoning (if, for example, Alexander’s doctors treated him with toxic hellebore) and for deliberate poisoning by his enemies. In 2009, Leo Schep, a toxicologist, considered what sort of toxin might have been administered if Alexander had been poisoned: Schep proposed hellebore. But the symptoms of hellebore poisoning were well known in antiquity and would probably have been recognized by Alexander’s doctors. Hellebore induces severe diarrhea, not mentioned by any of the ancient sources who described Alexander’s death.

In 2004, Paul Doherty concluded that Alexander was poisoned with arsenic, which might have preserved the body from decomposition. Graham Phillips (2004) suggested an alkaloid plant such as belladonna (deadly nightshade),aconite (monkshood)—both easily available—or strychnine (first suggested by Milnes 1978, 255–57, citing Theophrastus *History of Plants* 9.11.5–6)). Since Alexander did not suffer vomiting which always accompanies alcohol, belladonna, arsenic, and aconite poisoning, Phillips concluded that the agent must have been strychnine. Strychnine poisoning appears to match some of the symptoms: paroxysmal contractions of muscles followed by complete relaxation, skin very painful to touch, high fever, sweating, high blood pressure, intense thirst, and lockjaw. *Nux vomica* has a very bitter taste, however, and the plant comes from southern India. Moreover, poisoning by this
and other plant toxins would have required repeated doses to effect the slow death, which would increase the chance of the plot being discovered. It is also notable that Alexander, notoriously paranoid at this time, apparently did not suspect poisoning (Engels 1978, 224–25).

**Legendary Styx Poison Suspected**

Rumors of poisoning began to circulate among some of Alexander’s companions after his death (Justin 12.14; Plutarch *Alexander* 77.1–3; Diodorus 17.118). Many people had motives and means. Suspicion fell on his enemies Antipater, viceroy in Macedonia, and Antipater’s son Cassander, who had recently arrived in Babylon from Greece. Plutarch (75–77) gives a detailed account of the alleged conspiracy and the special poison. Some claimed that Aristotle, Alexander’s old friend and tutor, provided the Styx poison because he now feared his student (Arrian 7.24–27; Plutarch *Alexander* 77; Pliny 30.53; Dio Cassius 77.7, p III Boissevain (Caracalla); Johannes Zonaras *Annal.* 4.14, Migne 134, p 356C). M. Plezia (1948) and Ingemar Düring (1957, Testimonia 29a–e pp. 296–98) gathered and commented on the ancient evidence for Aristotle’s involvement, and suggest that the notion was debated in polemics about the Callisthenes affair by later peripatetic philosophers who defended or defamed Aristotle (Monte Johnson per corr July 2010).

Plutarch (*Alexander* 75–77) identifies the authority for implicating Aristotle as Hagnothemis, who heard it from Antigonus, a trusted contemporary of Alexander. The poison said to have been prepared by Aristotle was “deadly cold water from the rock cliff near Nonacris, gathered [or distilled] like a delicate dew [or exudation] and stored in an ass’s hoof, for all other vessels were corrupted by its icy, penetrating corrosiveness.”

Aristotle was in Athens at the time of Alexander’s death. Aristotle’s nephew Callisthenes had been murdered by Alexander in 327 BC, which was thought to be a reason for Aristotle’s distrust of Alexander. Was Aristotle also suspected because had written about Styx poison in a lost book of natural history? (We know that his colleague Theophrastus did write about Styx poison.) According to Arrian (7.24–27), some said that “Antipater sent Alexander medicine which had been tampered with and he took it, with fatal results. Aristotle is supposed to have prepared this drug. . . . Antipater’s son Cassander is said to have brought it. Some accounts declare that he brought it in a mule’s hoof, and that it was given to Alexander by Cassander’s younger brother Iolaus, who was his cup-bearer. . . . others state that Medius, Iolaus’ lover, had a hand in it, . . . it was Medius who invited Alexander to the drinking-party [where Alexander] felt a sharp pain after draining the cup.” The *Greek Alexander*
Romance (Stoneman 1991, 3.31-32) maintained that the banquet was a conspiracy involving Antipater, Cassander, Iolaus, and others.

The true cause of Alexander's untimely death more than 2,000 years ago will probably never be solved. Even if Alexander was poisoned, the story of a strange "icy dew" gathered from the banks of the Styx waterfall, the dismal entrance to Underworld, seems too fantastic to be genuine. Yet the descriptions of the poison and its source remained consistent in many different sources over many centuries. Moreover, it is striking that although some of the historians and naturalists doubted the reality of a poison plot, not one ancient writer casts doubt on the existence of a deadly substance from the Styx River. These features of the ancient evidence suggest that scientific detective work might reveal the identity of a potentially deadly poison from the Styx.

Notably, some of Alexander's symptoms and course of illness seem to match ancient Greek myths and folk traditions associated with the Styx— he even lost his voice, like the gods who fell into a coma-like state after drinking from the Styx. This similarity of symptoms may have led his companions and others to assume (or to claim for propaganda purposes) that Styx poison was responsible. Such a sacred pharmakon or drug would lend an aura of divinity to Alexander. For his friends, the idea that their great hero had succumbed to the ancient poison taken from the gods' sacred oath-river would have carried symbolic resonance. Moreover, a parallel ancient tradition associated the Styx with immortality. Here was a venerable drug worthy of ending the earthly life of their "semi-divine" leader.

As stated in the beginning, this paper does not argue for or against a poison plot. Our goals are, first, to learn whether the Styx could have harbored a deadly toxin recognized in antiquity; second, to identify the substance that was rumored to have poisoned Alexander; finally, to determine whether or not it was plausible to claim such a poison could have killed Alexander.

Naturally Occurring Deadly Toxins?

In antiquity the Styx was called 'dark" and 'black" and the modern name is "Black Water." Black could indicate poison and/or might refer to the apparent color of the water or the dark stains on the limestone bedrock around the stream (see FIG 2). Frazer noticed the black and red streaks caused by "a dark incrustation" on the rock "wherever it is washed by falling water or spray" (1913, 248-55).

Alexander von Humboldt (1860, 194-95) discussed the malevolent image
of the Styx in myth and its alleged role in Alexander’s death. He speculated that the “abnormally” cold temperature of the glacial water may have been “occasionally injurious to wanderers,” or that the inaccessibility and desolate site of the falls led to its “evil reputation.” But Humboldt also thought that that “pernicious qualities” derived from minerals in rock fissures may have poisoned the water in antiquity.

In 1913, Frazer (253) mentioned a “chemical analysis” of the Styx/Mavroneri that showed no harmful substances “held in solution.” Frazer does not give the date or name of the chemist. As far as we can discover, the only report of a chemical analysis of Styx water occurred in 1815, at a meeting of the Scandinavian Society. Professor Broendnave read “a memoir on the Styx and its sources near Nonakris in Arcadia,” followed by “a chemical analysis of the water taken from its springhead,” presented by Professor Hans Christian Oerstedt, the Danish chemist who discovered electromagnetism. Unfortunately, it seems that Oerstedt’s findings were not recorded (Literary Panorama 1814–1819, 83).

In 1922, James Reuel Smith visited the Styx/Mavroneri and described the hydrogeology of the mythic spring waters precipitating through karst limestone. “From the very high overhanging cliff face, a Spring of poisonous water dripped drop by drop upon a natural shelf of stone below it, and oozing through the shelf fell at intervals into the river that ran beneath. Those drops the Greeks called the Water of the Styx; they were deadly both to man and beast, so that to have ‘taken a draught of the Styx’ became one of the many early euphemisms for physical dissolution. The constant dripping wore a hollow in the shelf of stone large enough to accommodate some small fish that were as deadly as the water [as stated by Theophrastus, above]; and, to protect both the hungry and the thirsty from this death trap, the hollow was surrounded” with a stone wall, as reported by Herodotus (cited above).

Consistent ancient literary evidence suggests that something unique in the Styx/Mavroneri watercourse gave rise to its poisonous reputation. The stream could contain corrosive acids (sulphuric, carbonic, etc) or caustic, alkaline minerals (such as calcium carbonate). Both acid and base substances adversely affect metal and other materials. Ancient mining could release lethal minerals (eg, arsenic, zinc, cadmium, selenium) into rivers, as suggested by historian of geology Mott T. Greene (per cor June 2010). The “culprit could be arsenical copper, widely documented in Bronze Age Greece, and preferred because it made a harder bronze. Once smelted, the arsenic in the slag is easily oxidized and mobilized, and it could reach lethal proportions quickly.” But we have not found evidence for mining operations in Arcadia in antiquity. If the Styx/Mavroneri harbored poisonous substances, it is likely that they would have been
naturally occurring. There are several possible sources for natural toxins in the water or the stream banks.

Hydrofluoric acid mist has been suggested (Patrick Lawrence, per cor June 2010; mists were mentioned by some ancient authors). It dissolves glass and is extremely corrosive to metal. This substance is very rare, but it occurred in levels lethal to livestock and humans after the Laki volcano erupted in Iceland in 1783.

Toxic metallic salts can be carried by streams (Hughes 1994). Uranium and other radioactive elements have an affinity for caliche; uranium and radioactive deposits, as well as caliche, exist in Greece. Mineral-laden water and sulfuric acid can vent from hydrothermal fields (Clendenon 2009, 129). Arsenic is found in hot-spring fed waters in Greece (D'Alessandro et al 2008) and thermal venting areas along fault lines are common. The southern edge of the Gulf of Corinth lies along a fault, which could affect the limestone Chelmos mountain range (Higgins and Higgins 1996, 69–70).

So far, thermal features are unknown in the region of Mavroneri. However, Walter D'Alessandro, a hydro-geochemist who has studied Greek rivers and ground waters and is interested in the Styx legend, points out that “high concentrations of poisonous elements would not necessarily be related to thermalism” (2010) The dark red–black stains on the rock face at the falls could indicate iron oxides and hydroxides. Ferrous iron (iron sulphate, copperas or green vitriol) salts tend to oxidize and become non-water soluble “rust,” which might explain the stained bedrock (Henrik Lassen, per cor, June 2010). “Many toxic elements (such as arsenic) have a high affinity to these iron compounds,” notes D'Alessandro (2010). “The toxic elements can be either retained or released depending on changing physico-chemical parameters of the waters, mainly water acidity.”

D'Alessandro (2010) also wonders whether the gloomy reputation of the River of Death and the modern name Mavroneri (“Black Water”) could have been related to observations of dark–colored water due to the presence of abundant dissolved organic matter, deriving from the extensive forest and plant cover of the area.” After floods or during spring thaws, profuse decomposing vegetation swept into the water can make it appear black. This also could render the stream poisonous, since “many toxic elements have also high affinity to attach to dissolved organic matter” (D'Alessandro 2010).

In addition, the toxic plants themselves can poison streams after flooding. “Seasonal plant poisons in rivers pose a threat well known to
ranchers and herders. If a toxic plant, such as aconite or hemlock, is profuse along the stream, spring flooding or thaws can wash enough plant material into the stream to kill mammals (James Kirkland, per cor July 2010). Deadly plants in Greece include hemlock, aconite (monkshood), hellebore, yew, oleander, and euphorbias. Such seasonal poisonings could occur in many other rivers as well as the Styx/Mavroneri.

The Styx/Mavroneri may have hosted killer bacteria, unique to its ecosystem and geology. For example, as dead plants decompose in stagnant pools subject to evaporation, botulism can occur. In dry climates, botulism bacteria can remain viable in the soil or rock. Botulism does not bring fever, but causes neck and body paralysis, and inability to speak. But botulism and seasonal plant poisonings of the river would not be consistently or exclusively found in the Styx/Mavroneri (some ancient writers did described the Styx as sluggish, with stagnant pools).

We suggest that the notorious poison collected at the Styx waterfall was a soil–derived bacterium of exceptional lethality. Macrophytes, bryophytes, algae, cyanobacteria, and other organisms often colonize the surface of travertine and caliche, hard mineral crusts that form on limestone and on moss and lichen growing on limestone. Cyanobacteria can be neurotoxic, hepatotoxic, cytotoxic, and endotoxic at levels dangerous to humans and animals.

**Calicheamicin**

Our investigation focused on a lethal soil–drived microorganism, one that could thrive in the geological environment and climate of the Styx/Mavroneri watercourse. We suggest that the most promising source of the dread Styx poison was an *echinospora* species of Actinobacteria naturally occurring in the limestone terrain. This deadly species of spore–producing bacterium has only recently come to the attention of modern science.

In the 1980s, a toxicologist in Texas collected a sample of caliche and tested it in a laboratory. He discovered calicheamicin, a secondary metabolite of an extremely toxic, gram–positive soil bacterium (*Micromonospora echinospora*). The bacterium grows in caliche (calcrete, hardpan), the crusty white to rust–colored deposits of calcium carbonate that leaches or precipitates out of limestone, especially where water pools and then evaporates. Water that flows through limestone is charged with calcium carbonate, which deposits hardened crusts on rock surfaces; caliche also forms crusts and scale on any metal or clay materials. Layers of caliche can be several inches to several feet thick, on the surface or
underground. (FIG 5)

The geographical distribution of the pernicious bacterium is not yet known, but caliche is common in limestone karst landscapes, and it also encrusts moss at limestone springs. Mount Chelmos, the source of the springs that create the Styx/Mavroneri, is limestone. The geology of friable karst limestone and the arid climate of the Styx/Mavroneri falls region supports the formation of caliche, creating an environment where *Micromonospora echinospora* could have colonized caliche in antiquity. The poison could be gathered by scraping colonized crust from the limestone into a container for transport. Non-water soluble, calicheamicin would form a film or suspension in water. But it could be dissolved in alcohol.5

As far as we can ascertain, only the *echinospora* species produces calicheamicins. Calicheamicin gamma (1) is the predominant form. (FIG 6) Naturally occurring calicheamicin is an extraordinarily potent cytotoxin (cell-killer). It is one of the most cytotoxic substances known, with a cellular lethality greater than that of ricin or cyano morpholinyl anthracycline (Lode et al 1998).

Calicheamicin imparts its deleterious effects in mammals by causing irreparable double-strand breaks in DNA. These breaks ultimately lead to apoptosis (programmed cell death), multiple massive organ failure (liver, heart, kidney, bladder, lung, and nervous system), leading to death. Cytotoxic agents attack rapidly dividing cells such as bone marrow, intestinal epithelium, hair follicles, and epithelial cells of the trachea and esophagus. The signs and symptoms of poisoning are secondary to the malfunctioning of these cellular systems and massive organ failure. Symptoms of poisoning would include weakness and fatigue, swelling of mouth and throat, neurological and organ damage, and severe pain. Cytotoxins can also cause high fever, chills, and severe nerve and muscle pain. Death would be imminent, as there is no antidote once a lethal dose of calicheamicin is ingested.

Because it is 1,000 to 10,000 times more toxic to normal cells than any known anti-cancer drug, calicheamicin is too lethal to normal cells to be used in pure form in chemotherapies. Patients treated with cytotoxic agents often suffer from mouth and throat problems. Alexander lost the ability to speak (one source said his tongue was too swollen for speech). We note that his mortal illness became progressively worse over nearly two weeks. Indeed, depending on the dose, death from a cytotoxic substance would likely take several days due to the toxic mechanisms (DNA damage, liver and other organ failure, cell death, and so on).
Naturally occurring calicheamicin could cause a course of illness and death like that described for Alexander. Multiple doses would have been required for the plant poisons that have been suggested, whereas a single poisoning with a remarkably tiny amount of calicheamicin would be fatal. We propose that calicheamicin was responsible for the Styx/Mavroneris’s dreadful reputation in antiquity and that it was the fabled substance rumored to have killed Alexander the Great.

**Hoof or Horn Vessel?**

Many ancient writers claimed that the Styx substance was so cold and corrosive that it destroyed containers of all materials except an equine hoof or a special horn. Although the term “corrosive” is generally used for corruption of materials by either acid or alkali substances, technically “corrosive” refers to destruction of metal and other materials by strong acids or oxidizers, whereas “caustic” refers to destruction or “chemical burn” by strong alkali–base substances. Some minerals and salts along the Styx/Mavroneris streambed may be highly acidic corrosives, others may be caustic. The stream flows through limestone bedrock: lime (calcium oxide and calcium hydroxide) is base, non–acidic. Caustic limestone dust is a dangerous irritant to the eyes, skin, throat, and lungs. Alkaline caliche forms cement–like crusts on metal and degrades metal pipes and cables.

What accounts for the belief that only hoof or horn could withstand the poison from the Styx? Hooves and horns, like hair and claws, are keratin structures. (FIG 7) Is there something about the nature of keratin that made people believe that it would be an appropriate container to transport water or a substance from the Styx/Mavroneris stream?

Would a vessel of keratin be the most suitable for storing calicheamicin–bearing limestone crust? Keratin is a fibrous, waterproof, rigid, protein substance whose properties include high tensile strength and resistance to proteolysis (hydrolytic breakdown into soluble substances). It can be used as fertilizer because it is very slowly broken down by soil bacteria. It does not dissolve in cold or hot water. Strong acids and strong alkali substances can soften and eventually dissolve keratin.

According to Pseudo–Callisthenes (Wolohojian 1969), the hoof container was boiled. Mary Renault (1979, 260–61) suggests that this “remarkable detail” might refer to folk knowledge of boiling hooves—could the jelly that forms in the boiled hoof have served as a culture to preserve a deadly microbe? Gelatin comes from collagen from boiled animal bones and hides, whereas boiling hooves produces partially hydrolyzed keratin.
Adding an acid (such as vinegar) creates a thick gel, which has been used as glue since very early human times.

Further chemical analysis of the interaction of keratin and minerals from the Styx/Mavroneri, including caliche and calicheamicin, would be needed before the above questions can be answered. Scientific investigation might—or might not—reveal advantages to storing caliche/calicheamicin in a non–metallic container made of animal keratin.

On the other hand, magical thinking could account for the ancient notion that only a hoof or horn would withstand the poison from the Styx. In Greek mythology, the stamp of the hoof of a horse (often Pegasus) on rock was said to be the origin of some springs. But more significantly, the “primeval water” of the Styx (Hesiod *Theogony*) was sacred to the gods and possessed mysterious powers of life and death. Such a substance should only be contained in a natural vessel, not in a man–made container. In Greek myth, the goddess Iris carried Styx water in a cup of gold, a sacred incorruptible metal. But we note that for mortals at the Styx, various authors specify that vessels created from metals, glass, crystal, agate, and pottery were prohibited. Containers of metal, glass, clay, crystal, and agate are manufactured vessels, while hoof and horn are organic substances that are already shaped in the forms of natural, waterproof containers. Naturally occurring, non–man–made containers are advised for holding powerful pharmaka in other ancient texts. For example, the sorceress Medea gathered the deadly sap of a plant in a white seashell from the Caspian Sea, not in a manufactured vessel (Mayor 2010, 63 and 391n33). As we saw above, Theophrastus mentioned that the Styx water could be collected with a sponge on a stick, another natural method.

It is interesting to note the existence of at least two surviving red–figure Attic vessels (5th century BC) in the shape of a hoof (Richter 1939). The purpose and reason for the unusual shape is unknown. (FIG 8) Could these drinking cups be ironic allusions to the ancient lore about swearing oaths by the dangerous waters of the Styx, which could only be contained by a hoof?

Notably, the earliest surviving “scientific” discussion of Styx poison appeared in fragments of a lost work by Theophrastus, Aristotle’s pupil. After Alexander’s death in 323 BC, Aristotle left Athens, in fear for his life. His successor as director of the Lyceum in Athens in 323 BC was Theophrastus. As we have seen, rumors implicated Aristotle in the conspiracy to kill Alexander with the Styx poison sealed in a hoof. Pliny (30.53) even stated that Aristotle was the one who first discovered that the poison could be stored in a hoof: “Of all known substances, it is only
a mule's hoof that is not corroded by the poisonous waters of the Styx—a memorable discovery made by Aristotle, to his great infamy, on the occasion when Antipater sent some of this water to Alexander the Great, for the purpose of poisoning him.”

Interestingly, however, Theophrastus (cited above, cf Philo of Heraclea, fragment in Stobaeus) claimed the Styx corrupted all metals *as well as keratina*, horns and hooves. He contradicts the ancient traditions that the poison could only be contained in an equine hoof. The impression given by Theophrastus’s statement is that no known material could withstand Styx water. After Aristotle’s death, philosophers apparently debated his involvement (Plezia 1948; Düring 1957). Was Theophrastus responding to rumors that his teacher Aristotle was part of a plot to poison Alexander? Did Theophrastus intend to refute the rumor that Alexander could have been poisoned by water from the Styx? The notorious Styx poison and its relationship to the death of Alexander continues to raise more questions than answers.

**Conclusion**

Scientists have yet not looked for calicheamicin in Greece. But caliche is common in Greece’s arid, limestone-dominated landscape (Higgins & Higgins 1996, 50, 154, 206; Clendenon 2009, 223, 261–62). The geochemistry of the Styx/Mavroneri River has not been studied by modern hydrologists and chemists. The author are in correspondence with Italian hydrochemist Walter D’Alessandro, who hopes to carry out tests of the water and environment of the Mavroneri waterfall, perhaps in October 2011 (D’Alessandro et al 2008 and D’Alessandro 2010).

We propose three hypotheses:

- Calicheamicin was present in the Styx region in antiquity
- Its extraordinarily toxic, fatal effects were observed in antiquity
- Alexander’s death appeared to some to be caused by this poison

We may never know the real cause of Alexander’s death. He may have died of natural causes. Even if Alexander was poisoned, the agent may have been an easily available mineral or plant toxin such as aconite, rather than caliche bacteria scraped from Stygian limestone in Greece and transported to Babylon in a hoof. Yet, given that Alexander’s friends believed his symptoms fit those of fabled Styx poisoning, it may be instructive to compare Alexander’s symptoms to the known effects of calicheamicin poisoning. We submit that this toxin has close affinity to the course of Alexander’s reported symptoms and death, making it at
least as plausible an agent of poisoning as the aconite, arsenic, strychnine, or hellebore suggested by other scholars.

Modern scientific tests of the water, soil, and rock around the Styx/Mavroneri waterfall or source may or may not detect the current presence of mineral or organic toxins or bacteria. In either case, however, it would still be impossible to know whether such agents were present in antiquity or at the time of Alexander’s death. For the purposes of this investigation, we can say that the ancient literary evidence, taken together with modern toxicological knowledge and the geological conditions of the Mavroneri, suggests that the belief in a deadly poison at the Styx waterfall might have been based on accurate observations of natural phenomena.

To reiterate: whether such a poison was actually contemplated or used in a plot to murder Alexander remains unknown. But it is safe to say that if calicheamicin was ingested, the result would have been an agonizing death over several days, a course of events compatible with those described in the ancient sources recounting the death of Alexander.

WORKS CITED

Atkinson, John; Elsie Truter; and Etienne Truter. 2009. “Alexander’s Last Days: Malaria and Mind Games?” Acta Classica (1 January). http://www.thefreelibrary.com/Alexander's+last+days%3a+malaria+and+mind+games%3f-a0221920136


D’Alessandro, Walter. 2010. Personal Correspondence, 29 July: “We have at present no data on the water geochemistry of the area [of the Mavroneri]. . . . Obviously it would be very interesting to have analyses of the waters, sediments, and stains. We have at present no plans to get samples of the area, but in October 2011 there will be a hydrochemistry congress in Kalavrita (www.hydrogeocongress.gr/en) which is very close to the area. We will attend this congress and it would be an occasion to take samples from the area.”


Richter, Gisela. 1939. “Two Recent Acquisitions by the Metropolitan


Smith, James Reuel. 1922. *Springs and Wells in Greek and Roman Literature: Their Legends and Locations*. Putnam’s Sons.


FIGURES and MAPS

MAP. Left, Achaia (ancient Arcadia), Greece. Right, A = Mt Chelmos, source of the Styx/Mavroneri.
FIG 1. The mythic Styx, River of the Underworld, was a popular motif in European literature and art. Etching by Gustave Doré.
FIGS 2. Views of Mt Chelmos and the Styx/Mavroneri waterfall. Top photo, Klaas Kamstra, 2004; lower left, Bill Zogo, lower right R. Pone.
FIG 4. The poisoning of Alexander, woodcut, by the Petrarch-Master, ca 1520, perhaps based on J. Hartlieb’s popular translation of the Alexander Romance, 1444–1500s. Left, Antipater (or Aristotle?) prepares the poison. Right, Cassander’s sons deliver the poison: Philip secretly pours the poison from a horse’s hoof into the lid of the goblet behind the cupbearer/taster Iolaus’s back. Iolaus then serves the uncovered poisoned goblet to Alexander, who expires in the center. (The central royal figure with the spear may depict the paranoid, enraged Alexander killing a faithful friend, or perhaps represents the stabbing pain suffered by Alexander; see Barb 1971).
FIG 5. Caliche, or calcrete, forms on limestone, and can encrust moss (upper right).
FIG 6. Chemical structure of calicheamicin gamma (1).
FIG 7. Hoof and horn, natural organic containers of keratin.
FIG 8. Vases in the shape of hoof, fifth century BC, left, Naples Museum, right, Metropolitan Museum (Richter 1939).
We wish to thank Walter D’Alessandro, Mott T. Greene, Kemal Güneş, Monte Johnson, Prudence Jones, Kenneth Lapatin, Patrick Lawrence, John Ma, David Meadows, Josiah Ober, Leventis Pantelis, Christopher Seal, Stavros Sim, Helen South, Richard Stoneman, Guido Valle, and Philip Wexler for valuable comments and suggestions. Our preliminary findings were published in Mayor 2010, 69; and Mayor and Hayes 2010.

Plato and others remarked on the Styx’s “fearful powers” (*Phaedo* 112e) and some writers also mention noxious mists and vapors, and mention sluggish water and stagnant pools (*Ovid Metamorphoses* 3.290; 4.433; *Seneca Hercules Furens* 709; cf *Homer Iliad* 2.751–55; 8.369; 14.271; 15.35–37; *Odyssey* 5.185; 10.511–13; 15.37; *Apolloborus*. 1.2.5; *Apollonius of Rhodes* 2.191.

Five men close to Alexander wrote accounts of his death, which were lost but are cited by later surviving historians: Alexander’s bodyguard/friend Ptolemy, his admiral Nearchus, his secretary Eumenes, his chamberlain Chares, and his military engineer Aristobulus. Ancient surviving sources include Diodorus, first century BC; Plutarch, ca AD 100; Pliny and Quintus Curtius Rufus, first century AD; Arrian, Pausanias, and Justin, second century AD; Aelian, ca AD 200; the *History or Romance of Alexander* dates to about AD 250.

The *Alexander Romance* differs in describing the container for the poison, lead inside iron, rather than an equine hoof. A fourteenth century illustrated manuscript of the Greek Alexander Romance has miniatures of the poison transported in a lead pyxis from Greece to Babylon, and passed to Iolaus the cup-bearer, Alexander drinking from a glass goblet (thanks to Kenneth Lapatin). The motif of a feather coated with poison brings to mind the account of the poisoning of the Roman emperor Claudius (*Tacitus Annals* 12.56–58): when poisoned mushrooms prepared by the notorious poisoner Locusta failed to kill Claudius, his doctor Xenophon used a poisoned feather, ostensibly to make him vomit.

As far as we can determine, the toxicologist who discovered calicheamicin in the 1980s in Texas simply scraped a sample of caliche rock into a plastic bag, taking no particular precautions for transport or storage.