Mycoplasma agassizii disease risk assessment for Australia

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Image 1: Radiated tortoise (*Astrochelys radiata*) [credit Vladimír Motyčka]

ZOO AQUARIUM ASSOCIATION AUSTRALASIA

Contents

1. Executive summary

Mycoplasma agassizii is a known pathogen of land tortoises (family Testudinidae), in particular the host genus *Gopherus* (gopher and desert tortoises, native to southern USA). The pathogen causes upper respiratory tract (URT) disease in susceptible hosts, with persistent infection resistant to antimicrobial treatment. There is extensive published literature on *M. agassizii,* and it is perhaps the best studied pathogen of Testudinidae.

Mycoplasma agassizii is host specific, requires close contact for transmission and does not survive for long outside the host. A number of exotic Testudinidae, housed in Australian zoos, are known to be positive for *M. agassizii* by PCR test.

An extensive literature review found reports of infection with *M. agassizii* (with possible associated URT disease) in several species of Testudinidae. There are two unpublished reports of infection in the closely related family Emydidae in the USA. Other than these two reports, there are no reports and no evidence of infection with *M. agassizii* or *M. testudineum* occurring outside the Testudinidae. There is no evidence that chelonians other than Testudinidae (and possibly Emydidae) are susceptible to infection with *M. agassizii.* Neither of the families Testudinidae and Emydidae occur naturally in Australia and Australian native chelonians are distantly related to these taxa.

This risk assessment determined that there is a **negligible** risk that *M. agassizii* could be a threat to native chelonian species in Australia. No risk mitigation is considered necessary.

The risk to non-chelonians, including other native species, humans and domestic animals, is considered **negligible**. No risk mitigation is considered necessary.

This risk assessment determined that there is a potential risk of transfer of *M. agassizii* infection to currently uninfected Testudinidae within Australian zoos, with a risk of **moderate** consequences to the individual. Australian zoos may wish to adopt internal risk assessment and risk management measures to mitigate this potential risk.

In Australia, only licensed zoos are permitted to keep exotic reptiles. Notwithstanding, some exotic reptiles, likely including Testudinidae, are held illegally in Australia. Risk to (or from) exotic Testudinidae present in Australia, but held outside the zoo compartment, was not assessed during this process.

Increased testing, including sequential testing, and/ or inclusion of serological tests, may better inform the status of current infection and exposure in Testudinidae in Australia.

As a general recommendation, active disease surveillance, along with opportunistic and targeted collection of baseline health and disease data, is encouraged for all Australian free-ranging wildlife populations. This, along with full investigation of morbidity and mortality events in wildlife, will help to better understanding and preparedness for response to all and any emerging diseases.

2. Purpose of this document

This document is a desktop disease risk assessment to assess the potential risk of the bacterial pathogen *Mycoplasma agassizii* (or the closely related *M. testudineum)* to Australia.

Risk questions addressed:

1) a) What is the likelihood of native Australian fauna, domestic animals or humans **outside** the zoo compartment in Australia being ***exposed*** to *M. agassizii* (or the closely related *M. testudineum*) with the subsequent establishment and spread of the pathogen throughout Australia?

   b) What is the likely **consequence** of a species of native Australian fauna or domestic animal (or human), outside the zoo compartment in Australia, being **infected** by *M. agassizii* (or the closely related *M. testudineum*), following exposure to the pathogen?

2) a) What is the likelihood of exotic or native fauna (or zoo workers), present ***within*** the zoo compartment in Australia, being ***exposed*** to *M. agassizii* (or the closely related *M. testudineum*) with the subsequent establishment and spread of the pathogen?

   b) What is the likely consequence of exotic or native fauna (or zoo workers), present **within** the zoo compartment in Australia, being infected by *M. agassizii* (or the closely related *M. testudineum*) following exposure to the pathogen?

3. Background and context

Mycoplasma agassizii is a bacterial pathogen of land tortoises in the family Testudinidae. *Mycoplasma testudineum* is a closely related species of bacterium, which has been associated with disease in only two species of land tortoises (*Gopherus* spp.).

Testudinidae are a family of land tortoises (see front page image). Testudinidae are not native to Australia. Small numbers of individual Testudinidae, of a number of different species, reside in several Australian zoos, in almost all states and territories of Australia. Many of these individuals have been present within Australian zoos for years, or even decades. Only licenced exhibitors (zoos) are permitted to keep exotic reptiles in Australia. Notwithstanding, some exotic reptiles, likely including Testudinidae, are held illegally in Australia, by private entities.

A PCR test was recently developed by Murdoch University for this pathogen. Subsequent testing revealed *M. agassizii* infection in several individual Testudinidae resident in zoos in Australia (see *Occurrence in Australia*).

Very limited testing for *Mycoplasma* spp. has been undertaken in native chelonians (free-living or captive) in Australia. The susceptibility of Australian native chelonians to *M. agassizii* (or *M. testudineum*) has not been previously assessed.

Radiated tortoises (*Astrochelys radiata*) fall in the family Testudinidae. A group of radiated tortoises was imported into Australia in 2018 and underwent post-arrival quarantine in approved

arrangements in a registered zoo in Qld. During this time, several of the tortoises underwent PCR testing for *M. agassizii*, with positive results.

Animal Health Committee (AHC; www.agriculture.gov.au/animal/health/committees/ahc) determined that a risk assessment should be undertaken to investigate and document the potential disease risks associated with the presence, and possible establishment or spread of *M. agassizii* in Australia. They requested the Zoo and Aquarium Association (the Association; ZAA; www.zooaquarium.org.au/) to lead this project.

Dr Andrea Reiss, Regional Veterinary Officer with the ZAA, has extensive experience undertaking biosecurity import risk assessments (www.agriculture.gov.au/biosecurity/risk-analysis) associated with importation of zoo animals, and wildlife disease risk assessments associated with wildlife translocations and management (Jakob-Hoff *et al.* 2014). Wildlife disease risk experts at Wildlife Health Australia (www.wildlifehealthaustralia.com.au/) reviewed and provided comment on a draft of this document. The document was also provided to Animal Biosecurity at the Commonwealth Department of Agriculture and Water Resources (now Department of Agriculture, Water and the Environment) for review and comment.

Scope and limitations of this risk assessment

1. Scope is outlined in Section 2 "Risk questions addressed".
2. No entry risk assessment has been undertaken as *M. agassizii* is already present in Australia (in zoo chelonians).
3. Disease risk has only been assessed for *M. agassizii.* (Where relevant, information is provided on the closely related *M. testudineum*). Disease risk associated with other *Mycoplasma* species has **not** been assessed.
4. The information on captive chelonians in Australia is drawn from data held by the Zoo and Aquarium Association and is specific to ZAA-member zoos.
5. No data is available from non-ZAA zoos in Australia. There is no method by which the Zoo and Aquarium Association can access information on non-member zoos, either on their collections or their practices and there is no established avenue to collect data from non-ZAA member zoos in Australia. State and territory licensing authorities maintain information on licensed premises within their jurisdictions, but they do not provide this information to ZAA.
6. No data is available on captive chelonians held (illegally) outside zoos in Australia; there is no method by which the Zoo and Aquarium Association can access this information.
7. Disease risk to (or from) exotic species of chelonians illegally present in Australia (i.e. outside of licensed zoos) is unknown and has not been assessed.
8. It is assumed that all licensed zoo operations are compliant with existing biosecurity and containment regulations (which differ between jurisdictions).
9. It is assumed that existing containment regulations for exotic chelonians within Australian zoos (i.e. that only licensed zoos are permitted to hold exotic chelonians) will not be relaxed in the future.

4. Introduction

Taxonomy, terminology, biology and ecology of chelonians

(See also Appendix 1).

Chelonian is the term used to refer to all turtles and tortoises. The terms **turtle** and **tortoise** are generally interchangeable when discussing Australian species. The term terrapin is used outside Australia to refer to fresh-water species of Chelonian. All living turtles/ tortoises/ terrapins are in the class Reptilia, order Testudines. There are two suborders of chelonians: **Cryptodira** ('hidden-necked turtles') and **Pleurodia** ('side-necked' turtles), characterised by how they withdraw their neck into their shell.

The **Cryptodira** suborder has three living superfamilies, the Chelonioidea (sea turtles), Testudinoidea (land tortoises and pond turtles) and Trionychoidea (soft-shell turtles and relatives).

> **Land tortoises** (or "true" tortoises) all fall in the family **Testudinidae**, suborder Cryptodira, having a typical appearance of domed carapace and elephantine hind limbs (see front page image). There are **no** Testudinidae native to Australia. The nearest endemic land tortoises are those native to south-east Asia.
>
> **Emydidae** is the most closely related chelonian family to Testudinidae (in the same superfamily). This family comprises terrapins and pond turtles. Emydidae are native to the western hemisphere and there are **no** Emydidae native to Australia.
>
> All **marine turtles** fall into the suborder **Cryptodira**, within the families Chelonidae and Dermochelyidae.

Australia's native chelonians are 23 species of freshwater turtle and six species of marine turtle. All but one of Australia's native freshwater species belong to the family **Chelidae** in the suborder **Pleurodia**; this family is found only in Australasia and South America (see www.environment.nsw.gov.au/topics/animals-and-plants/native-animals/native-animal-facts/freshwater-turtles). There are no Testudinidae or Emydidae native to Australia.

The only Australian freshwater turtle species in the suborder **Cryptodira** is *Carettochelys insculpta* (pig-nosed or pit-shelled turtle), which is the monotypic member of the family *Carettochelydidae* (see http://reptilesofaustralia.com/turtles/turtles.htm#.W44U5egzaM8).

Summary: Australian native chelonians are taxonomically very distantly related to land tortoises (Testudinidae).

Chelonians housed in Australian zoos

In Australia, only licensed zoos are permitted to keep exotic reptiles. A large number of native and exotic chelonian species are housed in Australian zoos (29 different genera [10 native; 19 exotic] in ZAA zoos, 15 Jan 2019); this includes a number of exotic land tortoise species (family Testudinidae).

The Zoo and Aquarium Association maintains studbook information on all species of chelonians held in ZAA-member zoos in Australia. ZAA-member zoos hold 51 individual land tortoises, across 16 species (data compiled 15 Jan 2019; see Appendix 2). Land tortoises are held in ZAA zoos in every

state/ territory other than Tasmania. All land tortoises in ZAA-member zoos have either been imported from overseas or are the descendants of imported individuals.

There is no reliable information available on the number or species of exotic land tortoises housed in Australian zoos outside ZAA membership (see "Scope and limitations of this risk assessment, dot point 5"). Enquiries made by ZAA in late 2019 via the reptile-keeping "community" across Australia did not reveal, to the best of our collective knowledge, the presence of tortoises of the family Testudinidae held in Australian zoos outside ZAA membership.

Although only licensed zoos are permitted to keep exotic reptiles in Australia, it is widely accepted that a number of exotic reptiles are held (illegally) and without permit, by private individuals in Australia. The generally accepted knowledge is that the majority of these illegally held exotic reptiles are either snakes or lizards. Reptiles in Australian zoos are all obtained through approved permit and there is no unregulated passage of illegally held reptiles in Australia into the zoo compartment.

In summary it is likely that there may be land tortoises resident in Australia, outside ZAA-member zoos, however no data on this has been made available (see "Scope and limitations of this risk assessment; dot point 6").

A note on upper respiratory tract disease in chelonians

Upper respiratory tract (URT) disease is a commonly reported syndrome in land tortoises (Testudinidae) in the northern hemisphere. There is a proven association (causation) between infection with *M. agassizii* (and/or *M. testudineum*) and URT disease in North American desert tortoises (also called Agassiz's desert tortoise; *Gopherus agassizii* - see detailed information below) and other hosts of the genus *Gopherus*. Infection with *M. agassizii* has not been shown to cause URT disease in land tortoise species outside the genus *Gopherus*.

There are also many reports of URT disease in other species of land tortoises, where there is no accompanying evidence of *Mycoplasma* spp. infection (e.g. Blahak *et al.* 2004). Due to biology, physiology and environmental conditions, it appears that land tortoises often exhibit URT disease. Across the spectrum of all cases, it is likely there are multiple contributory and causal factors. *Pasteurella testudines*, iridovirus, herpesvirus, *Chlamydia* sp., several different fungi, nutritional deficiencies, and environmental irritants and allergens have all be reported as factors in URT disease in chelonians (Berry *et al.* 2002; Blahak *et al.* 2004; Boyer and Boyer 2006; Wendland *et al.* 2006). *Mycoplasma agassizii* is has been shown to be associated with (but not necessarily the cause of) some, but not all, cases of URT disease in Testudinidae. Outside the *Gopherus* host species where these pathogens have fulfilled infection trial requirements, there is little evidence that *M. agassizii* (or *M. testudineum*) consistently results in URTD in other Testudinidae hosts.

5. Epidemiology of *M. agassizii*

Aetiologic agent

Mycoplasma spp. are members of the class Mollicutes and the order Mycoplasmatales. They are the smallest free-living organisms. They have no cell wall but are bounded by a membrane (Stalheim 1990). Mycoplasmas are obligate parasites that only occur within a strict host association due to their requirement for many nutrients (Citti and Blanchard 2013).

Mycoplasma microorganisms are found in a wide range of living hosts. Many species of *Mycoplasma* are considered non-pathogenic and many have a commensal role in the mucosal surfaces of the host (Frey 2002). This is considered to be the case for many of the *Mycoplasma* species infecting chelonians (Berry *et al.* 2002).

Pathogenic *Mycoplasma* spp. have a predilection for mucosal surfaces where they localize and persist, protected by the fibrinous tissue reactions that characterize mycoplasmosis [disease due to mycoplasma infection] (Stalheim 1990). Pathogenic mycoplasma infections are often chronic in nature, with high morbidity and low mortality in the host. Infected hosts that do not display clinical signs (i.e. asymptomatic carriers) may be important in transmission of the pathogen. Pathogenic *Mycoplasma* spp. generally have a narrow host range and are adapted to a specific host species (one or sometimes several species) in which clinical disease is seen (Citti and Blanchard 2013).

Occasionally, secondary or "atypical" host species are colonised by a species of *Mycoplasma*. In the secondary host, signs of disease are rare, and if seen, are generally very mild (Frey 2002).

Virulence factors may result in differing levels of expression of disease from a *Mycoplasma* sp. within a given host species; this is believed to be the case for *M. agassizii* (McLaughlin 1997; Wendland *et al.* 2006; Citti and Blanchard 2013).

Mycoplasma agassizii (and *M. testudineum*) are known bacterial pathogens of the *Gopherus* genus of land tortoises (desert or ''gopher" tortoises, native to southern USA). In these host species, infection has been shown to cause upper respiratory tract disease ("tortoise mycoplasmosis") (Jacobson *et al.* 2014). The pathogens were first described by Brown *et al.* (1994) and Brown *et al.* (1995) when disease was seen in free-ranging desert tortoises (*Gopherus agassizii* and *G. morafkai*) in the United States. *Mycoplasma agassizii* has also been found in a wider range of land tortoise species (family Testudinidae), including species native to Europe. Its role as a potential pathogen in these species is far less clear (see further information below).

Mycoplasma agassizii is perhaps the best studied pathogen of Testudinidae and published literature is extensive. The pathogens, associated disease, investigation and epidemiology have recently been reviewed by Jacobson *et al.* (2014).

Agent properties

*Mycoplasma agassizii (*and *M. testudineum)* is a fastidious organism that grows slowly (2–6 weeks) at 30 °C in SP4 broth or agar (Brown *et al.* 1995; Brown *et al.* 2004; Citti and Blanchard 2013). *Mycoplasma* sp. lack a cell wall and are highly susceptible to desiccation in natural environments (Wendland *et al.* 2006). The organisms do not survive well outside the host.

Transmission (disease dynamics)

Mycoplasma agassizii is transmitted horizontally through respiratory secretions. Transmission pathways in the primary hosts (*Gopherus* spp.) have been well established by infection studies in desert tortoises and gopher tortoises (see Brown *et al.* 1994; Brown *et al.* 1995; McLaughlin 1997; Brown *et al.* 1999a; Wendland 2007). Transmission is via direct contact, notably when nasal discharge is present (Wendland and Brown 2019). Although transmission is considered more likely to occur when an individual has clinical disease, it has been suggested that, under appropriate

conditions, subclinically infected individuals may be able to transmit *Mycoplasma* spp. (Jacobson *et al.* 2014).

Close and prolonged contact between individuals, such as those that occur during courtship, mating, and agonistic behaviours, are considered requirements for transmission of *M. agassizii* and *M. testudineum*, in the primary hosts (Wendland *et al.* 2010).

Aerosol transmission is considered to be unlikely (Berry *et al.* 2002). A study in gopher tortoises showed that control animals, housed in pens adjacent to clinically affected tortoises, did not become clinically diseased nor did they seroconvert, suggesting that *M. agassizii* did not travel even relatively short distances over low (0.7 m) barriers (McLaughlin 1997).

Environmental transmission is considered very unlikely because of the susceptibility of *Mycoplasma* spp. to desiccation in natural environments (Berry *et al.* 2002). It is considered possible that in captive conditions, factors such as increased host density and higher loads of organic material could facilitate the persistence of the organism outside the host (Wendland *et al.* 2006). These risk factors can also be managed by appropriate stocking densities, hygiene regimes and by management of environmental conditions (such as temperature, humidity and pH) in the animals' enclosure. Factors which increase physiological stress (such as crowding, inappropriate temperature, poor nutrition and concurrent infections) may exacerbate expression of clinical disease in infected, captive tortoises (Wendland and Brown 2019). There is no evidence for vertical transmission and it is considered to be an unlikely mode of transmission (Jacobson *et al.* 2014).

Host response

A range of responses to *Mycoplasma* infection are known to occur in susceptible host species, from absence of disease to severe disease. In general, host immunological response plays a key role in the development of disease and expression of clinical signs across a wide range of taxa.

In Testudinidae, it is believed that differing strains of *M. agassizii* have differing pathogenicity, however there is currently no method available to assess the virulence of strains (Weitzman *et al.* 2017). It appears that that not all individuals from susceptible host species respond to *M. agassizii* infection with a severe inflammatory response, either because of differing strains of *M. agassizii* with variable pathogenicity or because of differing immune responses related to different host genotypes (McLaughlin 1997; Jacobson *et al.* 2014).

Development of an antibody response to *Mycoplasma* infection in experimental studies generally occurred 8 weeks post-infection (Brown *et al.* 2002). Recent studies indicate that an antibody response may not occur for more than 400 days post-exposure in susceptible species (Drake *et al.* 2019).

Infection with *M. agassizii* or *M. testudineum* in susceptible species (i.e. Testudinidae), once present, appears to be persistent (Jacobson *et al.* 2014; Wendland and Brown 2019).

Clinical signs and pathology

In susceptible species (Testudinidae, *Gopherus* spp.), infection can cause upper respiratory tract (URT) disease: nasal discharge, chronic rhinitis, conjunctivitis, ocular discharge and palpebral oedema (Wendland *et al.* 2006; Jacobson *et al.* 2014).

Infection may be present in susceptible species without clinical signs. Signs, when present, may occur intermittently, over periods of months or years (Wendland and Brown 2019). Subclinically infected animals may shed the organism and may show changes at a histopathological level (Wendland and Brown 2019).

Pathology primarily occurs within the nasal cavity, although pneumonia has occasionally been reported. Lesions in the nasal cavity may be focal or diffuse and range from mild to severe inflammatory changes, which include mucosal and submucosal hyperplasia with heterophilic and histiocytic infiltrates (Jacobson *et al.* 2014).

Infection with *M. testudineum* in susceptible species appears to cause similar but less severe, and more focal, pathology than *M. agassizii* (Jacobson and Berry 2012; Jacobson *et al.* 2014).

Diagnosis

Evidence of infection includes consistent clinical signs in known or suspected susceptible individuals of susceptible species. Typical histopathological changes are seen in samples collected during post mortem examination (Jacobson *et al.* 2014).

Two laboratory tests have been developed in an aid to diagnosis of mycoplasmosis in live chelonians. ELISAs have been developed in the USA to detect antibodies against *M. agassizii* and *M. testudineum* in plasma and serum of *Gopherus* spp. tortoises (Brown *et al.* 1999a; Brown *et al.* 1999b; duPre *et al.* 2011). The ELISA test is not available in Australia and will not be discussed further in this risk assessment.

Conventional and qPCR (specific for *M. agassizii*) have been developed in the USA and are now available in Australia (Brown *et al.* 1995; Brown *et al.* 2004; duPre *et al.* 2011). Samples for PCR include swabs of choana, nasal discharge or nasal flushes. The PCR test is considered highly specific. Sensitivity can vary as there is a risk of false negative results if the individual is not shedding or if sampling is inadequate (Jacobson *et al.* 2014).

Culture of the organism is difficult due to the fastidious nature of *Mycoplasma* spp. and can take up to 6 weeks for primary isolation. Nasal lavage is the most commonly used sample (Jacobson *et al.* 2014).

Treatment

Successful treatment of tortoises with mycoplasmosis is problematic, due to the characteristic agent properties and involvement of the host immune response in the expression of clinical disease. A range of systemic antibiotics suitable for *Mycoplasma* therapy have been used, along with supportive care and housing in the upper ranges of the individual's preferred body temperature range. There are no reports of confirmed long term success of antimicrobial treatment for mycoplasmosis in tortoises. Complete eradication of infection is considered unlikely in infected individuals, and relapse of shedding or clinical signs is often reported, some period after treatment ceases (even with prolonged courses of antibiotics) (Cowan 2018). At best, antimicrobial therapy may suppress clinical expression of disease and may suppress shedding of the organism by the host.

Once infection is confirmed in an individual, it should be considered to be persistently infected and a potential ongoing source of infection.

Prevention and control

Mycoplasma spp. do not survive for long outside the host. Standard infection control practices, including appropriate hygiene and disinfection, should be followed in all cases and in particular if animals are known or suspected to be infected.

Individual animals may be tested to determine their exposure status. Repeat PCR testing is advisable as shedding may be intermittent over weeks, months or years (Jacobson *et al.* 2014).

In susceptible species (*Gopherus* spp.), it is advisable to house known-infected animals separately from known-naive individuals in captivity, and to practice excellent hygiene when working with and between the two groups.

Captive animals should be maintained with excellent husbandry to reduce any physiological stress that may contribute either to acquisition of infection, or expression of clinical signs (which is also likely to increase their infectivity). Overcrowding, poor nutrition, inappropriate ambient conditions and high organic loads should be avoided in a captive environment (Wendland and Brown 2019).

Host species

There are numerous published reports of infection with *Mycoplasma* spp., or unspecified "mycoplasmosis" in chelonians. In many of these, the species of *Mycoplasma* has not been identified, or is not reported. Reports of infection include those associated with clinical disease and those where clinical disease is not present or is not reported. This risk assessment focuses on confirmed reports of *M. agassizii* in chelonians.

Mycoplasma agassizii infection has been reported in a wide range of free-living and captive land tortoise species in North America and Europe (Jacobson *et al.* 2014), Table 1.

Table 1: List of tortoise species in which *M. agassizii* has been detected (modified from Wendland *et al.* 2006), compiled March 2019, modified January 2020.

Common name	Species	Family	Reference
Desert tortoise (Agassiz's)	*Gopherus agassizii*	Testudinidae	Jacobson *et al.* (1991); Brown *et al.* (1994); Jacobson *et al.* (1995); Lederle *et al.* (1997)
Desert tortoise (Morafka's)	*Gopherus morafkai*	Testudinidae	Dickinson *et al.* (2001); Jacobson *et al.* (2014); Berry *et al.* (2015)
Texas tortoise	*Gopherus berlanderi*	Testudinidae	Guthrie *et al.* (2013)
Gopher tortoise	*Gopherus polyphemus*	Testudinidae	Beyer (1993); McLaughlin (1997); Brown *et al.* (1999a); Wendland (2007); Berish *et al.* (2010)
Leopard tortoise	*Stigmochelys (Geo.) pardalis*	Testudinidae	Jacobson *et al.* (1991); McArthur *et al.* (2002); Blahak *et al.* (2004)
Indian star tortoise	*Geochelone elegans*	Testudinidae	Jacobson *et al.* (1991), Blahak *et al.* (2004)
Radiated tortoise	*Astrochelys (Geo.) radiata*	Testudinidae	Jacobson *et al.* (1991); Blahak *et al.* (2004)
African spurred tortoise	*Geochelone sulcata*	Testudinidae	Blahak *et al.* (2004)
Hinge-back tortoise	*Kinixys* sp.	Testudinidae	Blahak *et al.* (2004)
Spider tortoise	*Pyxis arachnoides*	Testudinidae	Blahak *et al.* (2004)
Hermann's tortoise	*Testudo hermanni*	Testudinidae	Brown *et al.* (1999a); Blahak *et al.* (2004); Soares *et al.* (2004)
Afghan (Russian) tortoise	*Testudo (Agrionemys) horsfieldii*	Testudinidae	McArthur *et al.* (2002); Blahak *et al.* (2004); Soares *et al.* (2004)
Spur-thighed tortoise	*Testudo graeca*	Testudinidae	Jacobson *et al.* (1991); Blahak *et al.* (2004); Soares *et al.* (2004)
Marginated tortoise	*Testudo marginata*	Testudinidae	Blahak *et al.* (2004); Soares *et al.* (2004)
Egyptian tortoise	*Testudo kleinmanni*	Testudinidae	Blahak *et al.* (2004)
Red-footed tortoise	*Chelonoidis (Geochelone) carbonaria*	Testudinidae	Jacobson *et al.* (1991), Blahak *et al.* (2004)
Forsten's tortoise	*Indotestudo forstenii*	Testudinidae	Brown *et al.* (2001); Blahak *et al.* (2004)
Elongated tortoise	*Indotestudo elongata*	Testudinidae	Reported after testing zoo tortoises in Australia
Florida box turtle*	*Terrapene carolina bauri*	Emydidae	Siefkas *et al.* (1998); Rossell *et al.* (2002)
Red-eared slider*	*Trachemys scripta elegans*	Emydidae	Jacobson *et al.* (2014); E. Jacobson pers. comm. Jan 2019

* see further explanation below

Mycoplasma testudineum infection has only been reported in desert and gopher tortoises (*Gopherus* spp.) (Brown *et al.* 2004; Wendland 2007; Jacobson and Berry 2012; Jacobson *et al.* 2014; Wendland and Brown 2019). There is no evidence that *M. testudineum* has spread outside these two host species (Jacobson *et al.* 2014).

Significance of infection

Situation in North America

Mycoplasma agassizii infection has been confirmed to be the cause of disease only in a small number of land tortoise species. All these species are native to North America and all fall within the same genus:

- Agassiz's desert tortoises (*Gopherus agassizii*)
- Morafka's desert tortoises (*G. morafkai*, formerly falling within G*. agassizii*)
- gopher tortoises (*G. polyphemus*)
- Texas tortoises (*G. berlanderi*) [association only, not causality, shown].

Infection (with variable disease expression) has been detected regularly in free-living populations of these North American species. The origin of the pathogen is not known. Clinical signs are generally mild but infection with *M. agassizii* in these species is suspected to increase individual morbidity and mortality by hindering the host's ability to forage (due to a reduced sense of smell) and causing altered behaviours, possibly making a tortoise more susceptible to predation (Jacobson *et al.* 2014). Despite extensive study, there is no clear demonstration of a population level effect of mycoplasmosis in free-living *Gopherus* spp. (Sandmeier *et al.* 2009; Weitzman *et al.* 2017). We note it can be extremely difficult to demonstrate population level effects from disease in free-living wildlife species. Population level effects of *M. agassizii* have not been suggested in host species outside *Gopherus* spp.

Mycoplasma testudineum (in gopher and desert tortoises only) is associated with less severe and more focal pathology than *M. agassizii* (Wendland and Brown 2019).

Many earlier reports make poor distinction between infection with *Mycoplasma* spp. (which may be asymptomatic) and "mycoplasmosis" (which implies a disease state is present) (see for instance Brown D page 9 in Berry *et al.* 2002). In addition, in many reports, "*Mycoplasma*" is reported as being present, but the species of *Mycoplasma* is not identified. Subsequent publications have erroneously attributed infection to *M. agassizii*, when the original publication only identified *Mycoplasma* spp. e.g. Wendland *et al.* (2006) citing Berry *et al.* (2002). In some reports, a *Mycoplasma* species other than *M. agassizii* or *M. testudineum* has been identified e.g. novel *Mycoplasma* sp. identified in box turtles (Feldman *et al.* 2006; Farkas and Gál 2009).

Situation in Europe and other areas outside Australia

Several studies have reported *M. agassizii* infection in captive or wild Testudinidae in Europe (i.e. other than in *Gopherus* spp.) (McArthur *et al.* 2002; Blahak *et al.* 2004; Soares *et al.* 2004; Salinas *et al.* 2011). However, in many of the European reports of *M. agassizii* infection in Testudinidae, there was no evidence of clinical disease associated with infection, and/ or no causal link shown between infection with *Mycoplasma* and clinical signs exhibited.

One study of captive (privately held) *Testudo* spp. (family Testudinidae) in the UK confirmed presence of *M. agassizii* but found no statistically significant correlation between infection and clinical disease (although more infected animals had clinical signs than non-infected animals and signs were comparable with those seen with *M. agassizii* infection in North American Testudinidae) (Soares *et al.* 2004). Another study found European *Testudo* spp. with clinical signs of URT disease had low prevalence of both chelonian herpesvirus (chHV) and *Mycoplasma* spp. (including *M. agassizii*) and no correlation between infection and signs of URT disease (Salinas *et al.* 2011).

Another study examined three species of free-living European Testudinidae (*Testudo* spp.) that were temporarily held in captive arrangements (but sampled on arrival). They found strains of *Mycoplasma* spp. which were phylogenetically very close to (but not the same as) *M. agassizii* or *M. testudineum*. There was some evidence of clinical disease associated with infection but only in a relative small number of positive animals (n=3), making analysis difficult (Lecis *et al.* 2011).

Conclusion: Although there is some evidence that *M. agassizii* infection may be associated with URT disease in European and Asian species of Testudinidae, the association between infection and clinical disease (or pathological changes) remains far less clear than that demonstrated for American desert tortoises (*Gopherus* spp.) (Blahak *et al.* 2004). In the European and Asian species of Testudinidae, infection with *M. agassizii* may be one possible contributory factor in a multifactorial process resulting in signs of URT disease.

Reports of *M. agassizii* outside family Testudinidae

There are only two (both old) reports of *M. agassizii* infection outside the Testudinidae family, despite considerable study and testing for the pathogen across a wide range of chelonian species globally. There is one brief report of *M. agassizii* identified from a wild Florida box turtle (*Terrapene carolina bauri;* family Emydidae, closely related to Testudinidae) with URT disease (Siefkas *et al.* 1998). In the 20 years since this one report, there have been no further reports of *M. agassizii* infection in box turtles, despite ongoing testing. Recent studies encompassing wild and captive box turtles found a range of different *Mycoplasma* sp., but no evidence of *M. agassizii* (Feldman *et al.* 2006; Farkas and Gál 2009; Palmer *et al.* 2016; Jarred *et al.* 2018). At least some of the *Mycoplasma* sp. reported were considered likely to be commensal (Ossiboff *et al.* 2015). The original report of *M. agassizii* in the Florida box turtle is now considered likely to have been erroneous (and probably due to presence of a similar species of *Mycoplasma*) as no confirmatory sequencing was undertaken (J. Wellehan, Assoc. Professor Zoological medicine and microbiology, Uni. of Florida, pers. comm. Feb 2019).

There is one unpublished report of identification of *M. agassizii* by PCR in the lungs of red-eared sliders (*Trachemys scripta elegans;* family Emydidae) with pneumonia from Louisiana, USA (J. Roberts and E. Jacobson, unpublished data cited in Jacobson *et al.* 2014). Email correspondence with Elliott Jacobson (Professor, College of Veterinary Medicine, University of Florida; 9 January 2019) provided further information: the red-eared sliders were from a large turtle breeding facility (i.e. captive) and individuals had severe diffuse pneumonia. Multiple potential pathogens, including *M. agassizii,* were identified. In this instance, the role of *Mycoplasma* in disease expression is unclear, given the finding of several other pathogens, and the fact that *M. agassizii* is usually associated with URT disease, not pneumonia. It is not known if sequencing was undertaken in this instance to confirm the species of *Mycoplasma* detected. More recent studies in red-eared (and other) sliders have found other species of *Mycoplasma*, but have not found *M. agassizii*, despite testing (Silbernagel *et al.* 2013; Jarred *et al.* 2018).

To the best of our knowledge, there are no other reports of *M. agassizii* infection outside the family Testudinidae, despite extensive testing globally for this pathogen in a wide range of chelonian species. Given the numerous reports of new and novel *Mycoplasma* spp. in a range of chelonian species globally (including in Australia) e.g. Ossiboff *et al.* (2015), it is considered likely that many different *Mycoplasma* spp., are associated with different species of chelonians, including several

that are yet to be described. Many *Mycoplasma* spp. are likely to be commensal in chelonians, whilst some few may be shown to be associated with disease (Berry *et al.* 2002). Further work is required to better understand the implications of these findings in these hosts, however this is outside the scope of this risk assessment.

In conclusion, *M. agassizii* infection has been almost exclusively reported in chelonians from the family Testudinidae. Two unvalidated reports in the family Emydidae are not recent and are likely inaccurate as they have not been substantiated in more recent studies.

Only members of the *Gopherus* genus within family Testudinidae appear to be highly susceptible to *M. agassizii* infection. Koch's postulates have been fulfilled for *M. agassizii* infection and URT disease in two species of Gopherus, desert and gopher tortoises, and other members of this host genus (*Gopherus*) are clearly susceptible to disease as a result of infection with *M. agassizii.* There is limited evidence for an association between disease and infection in Testudinidae outside *Gopherus* spp. There is very little evidence that infection is significant in any species of chelonian outside the family Testudinidae (Jacobson *et al.* 2014; Wendland and Brown 2019).

Global distribution

Mycoplasma agassizii was originally reported from free-living *Gopherus* sp. of land tortoises in the USA. Subsequently, *M. agassizii* has also been described in several reports in captive and free-living land tortoises in Europe. It is likely that these pathogens moved from North America to Europe, and now Australia, due to human-assisted movement of reptiles around the globe. In Europe, studies indicate the likely presence of these pathogens in free-living Testudinidae, possibly as a result of practices of keeping mixed species of chelonians in the same housing and subsequently releasing animals into the wild without sufficient disease risk management.

Occurrences and testing in Australia

Captive Testudinidae in Australian zoos

Mycoplasma agassizii in known to be present in captive Testudinidae in a number of Australian zoos. Until recently, there was no ability to undertake molecular testing specifically for *M. agassizii* in Australia. A PCR test was developed (2016) by Dr Tim Hyndman at Murdoch University, WA (T.Hyndman@murdoch.edu.au). Use of this PCR revealed the presence of *M. agassizii* infection in several individual Testudinidae at least four licensed zoos in Australia. Testing has been limited. It is possible that the pathogen is present in a wider number of captive Testudinidae species, including ones held in other locations in Australia.

A group of radiated tortoises (*Astrochelys radiata;* family *Testudinidae*) were imported into Australia and underwent post-arrival quarantine in approved arrangements in a registered zoo in Qld during 2018. During this time, several of the tortoises underwent PCR testing for *M. agassizii*, with positive results.

Native Australian chelonians

To the best of our knowledge, the susceptibility of Australian native chelonians to *M. agassizii* (or *M. testudineum*) has not previously been assessed. Very limited testing for *M. agassizii* (or *Mycoplasma*

sp. in general) has been undertaken recently in native chelonians (free-living or captive) in Australia. A small number of captive western swamp tortoises (*Pseudemydura umbrina*; family Chelidae), native to Western Australia have been tested (negative results) for *M. agassizii* via PCR (Simone Vitali, Senior Vet Perth Zoo, pers. comm., Dec 2018). Bellinger River snapping turtles (*Myuchelys georgesi*; family Chelidae), endemic to NSW, were also tested (negative results) for *M. agassizii* via PCR, during investigations of a fatal disease outbreak (later attributed to a novel nidovirus) (Zhang *et al.* 2018).

It is known that *Mycoplasma* are, in general, highly host specific. Infection has not been confirmed in any chelonian species outside family Testudinidae[1]. *M. agassizii* appears to follow typical known epidemiology for pathogenic *Mycoplasma* spp., in that it is host-specific, with resulting mild or no disease in "secondary" hosts. All Australian native chelonians are taxonomically and genetically very distantly related to Testudinidae. Given this taxonomic distance and the high host-specificity of *Mycoplasma* in general and *M. agassizii* in particular, it would appear very unlikely that native chelonians would be susceptible to disease caused by infection with *M. agassizii* (or *M. testudineum*). We can find no reports of disease in Australian native chelonians similar to that seen in *Gopherus* spp. infected with *M. agassizii*.

6. Risk summary

- *M. agassizii* (and the closely related *M. testudineum*) are microorganisms known to cause upper respiratory tract disease in *Gopherus* spp. tortoises (land tortoises; family Testudinidae) in the USA.
- *M. agassizii* has only been detected in chelonians of the taxonomic family Testudinidae[2] . Neither Testudinidae nor Emydidae occur naturally in Australia. Representatives of both families are held in Australian zoos.
- *M. agassizii* appears to follow typical known epidemiology for pathogenic *Mycoplasma* spp., in that it is host-specific, with mild or no disease in "secondary" hosts.
- Infections in susceptible hosts tend to be chronic or life-long, with intermittent expression of disease.
- Transmission is via respiratory secretions and there is probably a higher risk of transmission during times when the host displays clinical signs and nasal discharge.
- The organism only survives for a limited time outside the host and close contact is necessary for transmission.
- Physiological stress likely plays a role in expression of clinical signs in susceptible individuals.
- Diagnosis in the living host is by PCR (available in Australia) and ELISA (not available in Australia), supported by known species susceptibility and clinical signs. Asymptomatic individuals may be infectious, but shedding is often intermittent, and negative PCR results should be interpreted with caution. Culture of nasal discharges is possible, but problematic.
- There is no vaccination available.

[1,2] Other than two unproven reports in Emydidae, discussed in detail in Section 5: "Reports of *M. agassizii* outside family Testudinidae"

- Treatment (with antimicrobials) has been described, but due to the nature of the disease, is problematic. There is no evidence that even extended antimicrobial therapy has permanently eradicated infection in susceptible individuals.
- There has been extensive testing of wild and captive chelonians in the northern hemisphere for *Mycoplasma* spp., including *M. agassizii* and *M. testudineum.*
- There is no substantive evidence that either *M. agassizii*[3] or *M. testudineum* can infect, or be carried by, chelonians outside the family Testudinidae.
- There is no evidence of *M. agassizii* or *M. testudineum* infection or carriage in any other taxonomic group (including other taxa of chelonians) and there is no evidence that either *M. agassizii* or *M. testudineum* are zoonotic.
- There has been limited testing of native Australian chelonians for *Mycoplasma* spp. including *M. agassizii.* There is no evidence of *M. agassizii* infection or carriage in native Australian chelonians.
- Native Australian chelonian species are all are distantly related to the known susceptible host species.
- *M. agassizii* is a well-studied pathogen and there have been numerous studies undertaken in northern hemisphere chelonians. Only members of the Testudinidae family appear to be genuinely susceptible to *M. agassizii* infection with resultant URT disease.
- In many reports in Testudinidae other than *Gopherus* spp., there is little association between infection and disease. Other than in *Gopherus* spp. hosts, it is likely that *M. agassizii* is only mildly pathogenic and/ or requires synergistic action from other pathogens/ causal factors for disease to occur.
- URT disease in chelonians (in particular Testudinidae) is common. There may be multiple and sometimes multifactorial causes, not clearly related to *Mycoplasma* infection, of URT disease.
- *Mycoplasma agassizii* is known to be present in multiple animals of different species of land tortoises (Testudinidae) in at least four different registered zoos in Australia, across three jurisdictions.
- Exotic reptiles may only be held under permit in licensed zoos in Australia. However, it is accepted that a number of exotic reptiles (primarily snakes and lizards) are held illegally by private entities in Australia. There is no unregulated movement of illegally held reptiles into Australian zoos.

7. Risk assessment

The likelihood of entry into Australia of *M. agassizii* was <u>not</u> assessed as the pathogen is already present in several Australian zoos. The likelihood of entry of *M. testudineum* was not assessed. As there is no evidence that *M. testudineum* is present in Australia, this hazard was not considered further in this risk assessment.

The likelihood of establishment of *M. agassizii* (across the two different compartments defined in the risk questions) was assessed against a six-point system. Consequence of establishment and spread was also assessed against a six-point system (see Appendix 3 for categories used and definitions). Establishment and consequence scores were entered into a standard risk assessment matrix to determine the overall risk (see Appendix 4).

[3] Other than two unproven reports in Emydidae, discussed in detail in Section 5: "Reports of *M. agassizii* outside family Testudinidae"

1) a) What is the likelihood of native Australian fauna, domestic animals or humans outside the zoo compartment in Australia being exposed to M. agassizii (or the closely related M. testudineum) with the subsequent establishment and spread of the pathogen throughout Australia?

b) What is the likely consequence of a species of native Australian fauna or domestic animal (or humans) outside the zoo compartment in Australia being infected by M. agassizii (or the closely related M. testudineum) following exposure to the pathogen?

The **likelihood** of native Australian fauna, domestic animals or humans outside the zoo compartment being exposed to *M. agassizii* (or *M. testudineum*) with the subsequent establishment and spread of the pathogen is assessed as **extremely low or negligible**.

Along with the extremely low or negligible likelihood of exposure, the **consequence** for native Australian fauna, domestic animals or humans **outside the zoo compartment** is assessed as **negligible.**

Using the table supplied in Appendix 3, the **overall risk** to animals and humans outside the zoo compartment is therefore assessed as **negligible.**

Rationale: *Mycoplasma* sp. are known to be host-specific. Transmission of this pathogen requires close contact between susceptible individuals. Fomite, environmental or aerosol spread are unlikely to be significant in transmission. Only chelonians in the two families Testudinidae (and, rarely, Emydidae) have been shown to be infected with these pathogens, despite extensive studies. Neither of these families occur naturally in Australia, and Australian native chelonians are distantly related to these taxa. There is no evidence to suggest that Australian native chelonian species would be susceptible to *M. agassizii (*or *M. testudineum)*. Australian native chelonian species are not typically housed with Testudinidae species in Australian zoos, as their physiology, husbandry and environmental requirements are extremely different.

Exotic chelonians housed in Australian registered zoos are held in secure, purpose-build enclosures within secure facilities. Individual exotic chelonians may be purposefully moved between registered zoos but do not leave the zoo "compartment". Due to the closed nature of zoo operations in Australia, there is no opportunity for exotic chelonians housed in Australian zoos to have direct contact with free-living Australian native chelonians (see AUSVETPLAN Zoos Enterprise Manual www.animalhealthaustralia.com.au/wp-content/uploads/2015/09/ZOO3.0-11-FINAL1Oct14-1.pdf).

Following this rationale, no other taxa (native fauna, feral or domestic species or humans) are considered at risk in any way from *M. agassizii (*or *M. testudineum).*

2) a) What is the likelihood of exotic or native fauna (or zoo workers) present within the zoo compartment in Australia being exposed to M. agassizii (or the closely related M. testudineum) with the subsequent establishment and spread of the pathogen?

b) What is the likely consequence of exotic or native fauna (or zoo workers) present within the zoo compartment in Australia being infected by M. agassizii (or the closely related M. testudineum) following exposure to the pathogen?

The likelihood of **exotic or native fauna (or zoo workers)** within the zoo compartment in Australia being **exposed** to *M. agassizii* (or *M. testudineum*) with the subsequent **establishment and spread** of the pathogen is dependent on the exposed taxon and the opportunities for direct contact with infected Testudinidae (see below)**.** The **consequences** of exposure for species within the zoo compartment will vary according to the exposed taxon and their assumed susceptibility to disease from this pathogen.

For individuals in the family Testudinidae, housed in close contact with infected and shedding individuals, it is **moderate** to **highly likely** that some non-infected, susceptible individuals will be exposed to and acquire infection. The **consequences** of infection for a susceptible individual are likely to vary with the individual from **very minor to moderate**.

Rationale: Severe disease has only been clearly associated with *M. agassizii* in hosts of the genus *Gopherus.* No members of this genus are currently held in ZAA-member zoos and no ZAA-member zoos indicate an interest in acquiring this genus. Different strains of *M. agassizii* appear to be related to differing levels of clinical expression of disease. There is currently no method to determine the pathogenicity of strains *M. agassizii* present in Australian zoo Testudinidae and different strains of differing virulence may be present. There is a **low** likelihood of infected hosts shedding large amounts of infectious organism (given the lack of *Gopherus* spp. hosts in Australian zoos[4]), and therefore the exposure risk is lower than it might be if infected *Gopherus* spp. hosts were present in Australian zoos. Nevertheless, overall there is a **moderate to high likelihood** that some individual Testudinidae will acquire infection and subsequently develop disease primarily associated with *M. agassizii (*if no risk management measures are implemented). The **consequence** of infection for the **individual** Testudinidae are likely to be **insignificant to moderate** (for example a range from minor, intermittent URT signs to moderate URT impacting individual welfare and overall health). The **consequences** to the population, zoo, species or wider community) are likely to be **minor**. The **overall risk** to individuals in the family Testudinidae within zoos was assessed as **low**[5].

For species within the **family Emydidae** within zoos the likelihood of **establishment and spread** of *M. agassizii* was assessed as **extremely low***.* The **consequence** for individuals and populations in family Emydidae within zoos was assessed as **very minor**. The **overall risk** for individuals and populations within the family Emydidae within zoos was assessed as **negligible**.

For all other species outside the families Testudinidae and Emydidae (including humans) the likelihood of **establishment and spread** of *M. agassizii* was assessed as **negligible** (as there is no evidence that infection can occur in other chelonian taxa). In addition, any **consequence** of exposure in this group was assessed as **insignificant** (as the risk of disease occurring in species not closely related to the primary host is considered very unlikely in *Mycoplasma* spp.) The **overall risk** for all other species outside the families Testudinidae and Emydidae (including humans) was assessed as **negligible**, following the rationale outlined above.

[4] Based on information available to the Zoo and Aquarium Association
[5] Taking the highest scoring across the different scenarios discussed in this section

8. Risk management measures

No risk management is considered necessary for the majority of scenarios examined in this document.

The overall risk for species within the family Testudinidae within zoos was assessed as **low** and risk management measures for this scenario may be warranted. Risk management recommendations are outside the scope of this risk assessment, however Australian zoos that hold Testudinidae may wish to adopt appropriate risk management measures, which might include:

- sequential PCR testing of individuals for *M. agassizii*
- maintenance of closed collections of Testudinidae until *M. agassizii* status is determined
- appropriate investigation and treatment of any illness in collection chelonians
- appropriate post-mortem investigation of any deaths in collection chelonians and
- appropriate biosecurity measures (including enclosure and worker hygiene practices) with all species, including chelonians.

More information can be found in the National Zoo Biosecurity Manual (Reiss and Woods 2011).

9. Conclusions

Mycoplasma agassizii is a known pathogen of land tortoises (family Testudinidae), in particular the genus *Gopherus* (native to the USA). The pathogen causes upper respiratory tract disease in susceptible hosts, with persistent infection resistant to antimicrobial treatment. *Mycoplasma agassizii* is host specific, requires close contact for transmission and does not survive for long outside the host. There is no evidence that chelonians other than Testudinidae (and possibly Emydidae) are susceptible to infection with *M. agassizii* (or *M. testudineum).* There is no conclusive evidence that infection with *M. agassizii* (or *M. testudineum)* results in disease in host species other than *Gopherus* spp. A number of exotic Testudinidae, housed in Australian zoos, are known to be positive for *M. agassizii* by PCR test.

This risk assessment found a **negligible** risk that *M. agassizii* could be a threat to native chelonian species in Australia. No risk mitigation is considered necessary. The risk to non-chelonians, including humans, other native species and domestic animals, has been assessed as **negligible**. No risk mitigation is considered necessary.

There is a potential risk of transfer of *M. agassizii* infection to currently uninfected Testudinidae in Australian zoos. Australian zoos may wish to adopt internal risk assessment and risk management measures to mitigate this potential risk. Increased testing, including sequential testing, and/ or inclusion of serological tests, may better inform the status of current infection and exposure in zoo Testudinidae in Australia.

In general, increased surveillance and testing of native chelonians for all *Mycoplasma* spp. (including novel species) is recommended, to improve understanding of these organisms.

As a further general recommendation, active disease surveillance, along with opportunistic and targeted collection of baseline health and disease data, is encouraged for all Australian free-ranging wildlife populations. This, along with full investigation of morbidity and mortality events in wildlife, will help to better understanding and preparedness for response to all and any emerging diseases.

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Appendix 1: Taxonomy of chelonids mentioned in this document

Common Name	Scientific Name	Genus	Family	Sub order	Native or exotic
Box turtle	*Terrapene carolina*	*Terrapene*	Emydidae	Pleurodira	exotic
Ornate box turtle	*Terrapene ornata*	*Terrapene*	Emydidae	Pleurodira	exotic
Red-eared slider	*Trachemys scripta elegans*	*Trachemys*	Emydidae	Pleurodira	exotic
Radiated tortoise	*Astrochelys radiata*	*Astrochelys*	Testudinidae	Cryptodira	exotic
African spurred tortoise	*Centrochelys sulcata*	*Centrochelys*	Testudinidae	Cryptodira	exotic
Chaco tortoise	*Chelonoidis chilensis*	*Chelonoidis*	Testudinidae	Cryptodira	exotic
South American yellow-footed tortoise	*Chelonoidis denticulate*	*Chelonoidis*	Testudinidae	Cryptodira	exotic
Red-footed tortoise	*Chelonoidis (Geochelone) carbonaria*	*Chelonoidis*	Testudinidae	Cryptodira	exotic
Indian star tortoise	*Geochelone elegans*	*Geochelone*	Testudinidae	Cryptodira	exotic
African spurred tortoise	*Geochelone sulcata*	*Geochelone*	Testudinidae	Cryptodira	exotic
Desert tortoise (Agassiz's)	*Gopherus agassizii*	*Gopherus*	Testudinidae	Cryptodira	exotic
Desert tortoise (Morafka's)	*Gopherus morafkai*	*Gopherus*	Testudinidae	Cryptodira	exotic
Texas tortoise	*Gopherus berlanderi*	*Gopherus*	Testudinidae	Cryptodira	exotic
Gopher tortoise	*Gopherus polyphemus*	*Gopherus*	Testudinidae	Cryptodira	exotic
Elongated tortoise	*Indotestudo elongata*	*Indotestudo*	Testudinidae	Cryptodira	exotic
Forsten's tortoise	*Indotestudo forstenii*	*Indotestudo*	Testudinidae	Cryptodira	exotic
Bell's hinge-back tortoise	*Kinixys belliana*	*Kinixys*	Testudinidae	Cryptodira	exotic
Spider tortoise	*Pyxis arachnoides*	*Pyxis*	Testudinidae	Cryptodira	exotic
Leopard tortoise	*Stigmochelys pardalis*	*Stigmochelys*	Testudinidae	Cryptodira	exotic

Spur-thighed tortoise	*Testudo graeca*	*Testudo*	Testudinidae	Cryptodira	exotic
Hermann's tortoise	*Testudo hermanni*	*Testudo*	Testudinidae	Cryptodira	exotic
Horsfield's (Afghan or Russian) tortoise	*Testudo (Agrionemys) horsfieldii*	*Testudo*	Testudinidae	Cryptodira	exotic
Marginated tortoise	*Testudo marginata*	*Testudo*	Testudinidae	Cryptodira	exotic
Egyptian tortoise	*Testudo kleinmanni*	*Testudo*	Testudinidae	Cryptodira	exotic
Pit-shelled turtle	*Carettochelys insculpta*	*Carettochelys*	Carettochelyidae (Subfamily Carettochelyinae)	Cryptodira	native
Bellinger River snapping turtle	*Myuchelys georgesi*	*Myuchelys*	Chelidae (Subfamily Chelodininae)	Pleurodira	native
Western swamp tortoise	*Pseudemydura umbrina*	*Pseudemydura*	Chelidae (Subfamily Pseudemydurinae)	Pleurodira	native

Appendix 2: Testudinidae in Zoo and Aquarium Association member zoos (Jan 2019)

Species	**Number of individuals**
Aldabra giant tortoise *Aldabrachelys gigantea*	**9**
Radiated tortoise *Astrochelys radiata*	**5**
African spurred tortoise *Centrochelys sulcata*	**2**
Chaco tortoise *Chelonoidis chilensis*	**1**
South American yellow-footed tortoise *Chelonoidis denticulate*	**1**
Galapagos tortoise *Chelonoidis nigra*	**3**
Star tortoise *Geochelone elegans*	**9**
Elongate tortoise *Indotestudo elongata*	**2**
Bell's hinge-back tortoise *Kinixys belliana*	**2**
Burmese brown tortoise *Manouria emys*	**1**
Painted wood turtle *Rhinoclemmys pulcherrima*	**1**
Leopard tortoise *Stigmochelys pardalis*	**7**
Spur-thighed tortoise *Testudo graeca*	**3**
Hermann's tortoise *Testudo hermanni*	**1**
Western Hermann's tortoise *Testudo hermanni robertmertensi*	**3**
Horsfield's tortoise *Testudo horsfieldii*	**1**
Total	**51**

Appendix 3: Likelihood and consequence categories and definitions

Table 2 Categories and definitions of likelihoods

Likelihood	Descriptive definition	Indicative range
High	The event would be very likely to occur	0.7 < to ≤ 1
Moderate	The event would occur with an even likelihood	0.3 < to ≤ 0.7
Low	The event would be unlikely to occur	0.05 < to ≤ 0.3
Very low	The event would be very unlikely to occur	0.001 < to ≤ 0.05
Extremely low	The event would be extremely unlikely to occur	0.000001 < to ≤ 0.001
Negligible	The event would almost certainly not occur	0 < to ≤ 0.000001

(Modified from Draft pest risk analysis for brown marmorated stink bug (*Halyomorpha halys*) DAWR 2017)

Table 3 Categories and definitions of consequence

Consequence	Descriptive definition
Insignificant	Isolated impact on individual animals at a single location or in a single population.
Very minor	Limited animal illness &/or deaths at a single location or population. Individual morbidities and/or mortalities but no measurable decline in population numbers. Only one host species affected.
Minor	Limited animal illness &/or deaths at one or more locations or populations. Possible individual morbidities and/or mortalities but little decline in population numbers. More than one host species possibly affected.
Moderate	Some animal illness &/or deaths at multiple locations or in multiple species. Small to moderate population level effects.
Major	Considerable animal illness &/or deaths at multiple locations, (or populations) or in multiple species. Major population level effects in one or more species or a moderate population decline of one species.
Extreme	Significant animal illness &/or deaths in multiple locations and species. Significant population declines of >80%, including possibility of extinctions, of one or more species. Widespread ecological or economic consequences.

(Adapted from NSW DPI Risk Assessment Template Table 1 - Consequence description for areas of impact; supplied)

Appendix 4: Risk assessment matrix – overall risk

(Note: Entry likelihood or consequence is not assessed)

Likelihood of hazard establishment and spread	Consequences of hazard establishment and spread					
	Insignificant	**Very minor**	**Minor**	**Moderate**	**Major**	**Extreme**
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

(Adapted from Department of Agriculture and Water Resources Biosecurity Import Risk Analysis Guidelines 2016)