**Patterns of infection, origins and transmission of ranaviruses among the ectothermic vertebrates of Asia**

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**Abstract**

Ranaviral infections, a malady of ectothermic vertebrates, are becoming frequent, severe, and widespread, causing mortality among both native and cultured species, raising odds of species extinctions and economic losses. This turn of events is possibly due to the broad host range of ranaviruses and the transmission of these pathogens through regional and international trade in Asia, where outbreaks have been increasingly reported over the past decade. Here we focus attention on the origins, means of transmission, and patterns of spread of this infection within the region. Infections have been recorded in both cultured and wild populations in at least twelve countries/administrative regions, together with mass die-offs in some regions. Despite the imminent seriousness of the disease in Asia, surveillance efforts are still incipient. Some of the infections transmitted within Asia may transmit across host-taxon barriers, posing a significant risk to native species. Factors such as rising temperatures due to global climate change seem to exacerbate ranaviral activity, as most known outbreaks have been recorded during summer; however, data are still inadequate to verify this for Asia. Import risk analysis, using protocols such as Pandora+, pre-border pathogen screening, and effective biosecurity measures, can be used to mitigate introduction to uninfected areas and curb transmission within Asia. Comprehensive surveillance using molecular diagnostic tools for ranavirus species and variants will help in understanding the prevalence and disease burden in the region. This is an important step towards conserving native biodiversity and safeguarding the aquaculture industry.

**Keywords:** Ranavirosis, emerging diseases, animal trade, biosecurity, seasonality

**Introduction**

Asia has been identified as an epicenter of emerging infectious diseases (EIDs) that display a complex relationship between host populations and the environment, involving domestic animals, wildlife, and human populations (Yadav *et al.,* 2020). Ranaviruses are pathogenic agents capable of infecting all classes of ectothermic vertebrates, causing significant morbidity and mortality depending on the specific virus, host, and environmental factors (Gray and Chinchar，2015). Given the broad host range and propensity to spread rapidly through regional and international trade, ranaviruses could contribute to species declines and pose a significant threat to cultured species and wildlife (Brunner *et al.,* 2015).

Here we outline the general background of the disease in the world in terms of host range, major outbreaks and severity, population declines and recovery, optimal climate, and diagnostic techniques. We then assess patterns of infection, origins and transmission of ranavirus infections in Asia while concentrating specifically on the following: (1) we compile recorded ranavirus infections by country, highlighting clinical presentation in hosts, seasonality, presence of mass die-off events, whether hosts are wild or cultured species, native or alien, their likely origins and the diagnostic tools used; (2) we evaluate potential routes of entry of ranavirus to Asia through cultured species; (3) we infer how climate change may affect ranavirus transmission dynamics in Asia; (4) we present a set of recommendations for inhibiting further ranavirus transmissions to hitherto unaffected parts of Asia, and measures to strengthen biosecurity. We hope thereby to enhance understanding of ranavirus species, evaluate the disease burden, help quantify risk, and identify host-pathogen interactions, epidemiology and conservation concerns, in the context of the economic importance of the disease in Asia.

**Discovery of ranaviruses, host range and outbreaks**

*Ranavirus* is one of the seven genera within thefamily *Iridoviridae* (International Committee on Taxonomy of Viruses, 2021a), a pathogen that infects infect ectothermic vertebrates such as bony fish, amphibians and reptiles. The diseases resulting from infections by species of *Ranavirus* (hereafter, ranaviruses)are characterized by a short incubation period and high mortality (Allender, 2018), affecting the health of both free-ranging and captive populations. Seven species of *Ranavirus* have been described according to the updated classification of the International Committee on Taxonomy of Viruses (ICTV): *Ambystoma tigrinum virus* (ATV)*, Common midwife toad virus* (CMTV)*, Epizootic haematopoietic necrosis virus* (EHNV)*, European North Atlantic ranavirus* (LfRV)*, Frog virus 3* (FV3)*, Santee-Cooper ranavirus* and *Singapore grouper iridovirus* (SGIV). Ranavirus isolates are considered members of the same viral species if they share >95% amino acid identity. However, *ranavirus maximus, cod iridovirus* and *short-finned eel virus* are potential new species that remain unclassified(International Committee on Taxonomy of Viruses, 2021b). The earlier classification, however, included *Frog virus 3* (*FV3*), *Ambystoma tigrinum virus* (*ATV*), *Bohle iridovirus* (*BIV*), *Epizootic hematopoietic necrosis virus* (*EHNV,* *European catfish virus* (*ECV*), and *Santee-Cooper ranavirus* (*SCRV*)(Jancovich *et al., 2012*). Among these, *Frog virus 3,* the type species of *Ranavirus*, was discovered originally in northern leopard frogs (*Lithobates pipiens*; Granoff *et al.,* 1965) and it is now known to infect at least 175 species across 52 families of ectothermic vertebrates on all continents except Antarctica (Duffus *et al.,* 2015). The *Frog virus 3* group are restricted to amphibian hosts, whereas ATVs are predominantly fish specialists that made a single switch to caudate amphibians (Price *et al.,* 2017). Further, CMTV-like ranaviruses may circulate independently in amphibian and fish communities. By 2015, ranaviruses had been reported in at least 105 species of amphibians in 18 families across 25 countries (Duffus *et al.,* 2015).

Most cases of ranavirosis have been recorded in North America, Europe, and Australia, with relatively few reported from Asia and Africa (Duffus *et al.,* 2015). This suggests that the intensity of the disease burden in Asia and Africa may be poorly understood and hence underreported. Outbreaks of FV3 and FV3-like viruses in amphibians have been detected in many different species of anurans and urodeles around the world: 42 amphibians from 9 species (an overall prevalence of 16.6% ) tested positive for ranaviruses among 253 amphibians belong to 20 species inCosta Rica (Whitfield *et al.,* 2013); FV3-like ranaviruses from five amphibian species in Europe (Gray and Chinchar, 2015); together with FV3 infection in wild three Spine Stickleback fish in eastern continental USA (Mao *et al.,* 1999a). The ability of ranaviruses to infect multiple host species is indicative of their high susceptibility, which leads to drastic population declines and eventually, the possible extinction of some host species (Earl and Gray,2014; Price *et al. 2014*). Alpine Newts (*Mesotriton alpestris*) and Common Toads (*Bufo bufo*) in Europe experienced substantial population declines (Price *et al*., 2014). Ranavirus die-off sites indicate up to 80% declines in common frog abundance in England (Teacher *et al.,* 2010). In addition, at sites where ranavirus die-offs occurred, amphibian recruitment attenuated in consecutive years (Petranka *et al.,* 2003), signifying the poor recovery of populations that have suffered declines. The variation, from no apparent mortality to massive die-offs, is one of the most intriguing features of ranavirosis outbreaks (Gray *et al.,* 2009).

Environmental factors are important in disease outbreaks, however, and anthropogenic stressors seem to have a stronger impact. For instance, the prevalence of ranaviral infection in green frog (*Lithobates clamitans*) populations has been shown to increase with proximity to industry and housing, although the underlying mechanisms were unclear (Daszak *et al.,* 2001; St-Amour *et al.,* 2008). Use of wetlands by cattle, which may negatively affect water quality, has also been suggested to stress resident tadpole populations (Gray *et al.*, 2007). It is believed that the stress, which is anthropogenically induced, could increase the pathogen prevalence in amphibian populations by compromising immunity. Water pollutants associated with unconventional oil and gas extraction (UOG) is reported to reduce weight in the metamorphic stages of *Xenopus laevis,* leading to a weakened antiviral immune response to the FV3 ranavirus (Robert *et al.,* 2019). Waterborne corticosterone (CORT), which is known to be immunosuppressive, was found to be positively correlated with ranavirus infection load in larval western tiger salamanders (*Ambystoma mavortium*; Davis *et al.,* 2019). Salinity related stress also is known to reduce tolerance to infection, increasing mortality in amphibians (Hall *et al.,* 2020).

**Seasonality**

The present understanding of the seasonality of ranavirus diseases derives mostly from studies outside the Asian region. In amphibians and fish, there is usually a rapid onset of ranaviral epidemics in the mid-to-late Northern Hemisphere summer, while outbreaks among reptiles seem to be irregular. Two distinct seasonal patterns of die-offs in farmed and wild populations of amphibians, fish, and reptiles attributed to ranavirus have been observed (Brunner *et al.,* 2015). The first shows rapid onset of die-off, usually occurring in fish and amphibians during summer months. The second shows variable vulnerability of host populations, ranging from absence of mortality to near-complete die-offs. These patterns are explained by four non-mutually exclusive hypotheses. First, there could be a detection bias, especially in wild populations, with some mortality events going unnoticed. Second, the rapid onset and seasonality of ranavirus die-offs may be due to the underlying epidemic dynamics following the introduction of ranavirus into populations earlier in the year. Third, in the summer months, hosts may become more susceptible to ranavirus infections because of seasonality associated with their reproduction (e.g., the prevalence of larvae). Finally, rising temperature during summer may trigger ranavirus outbreaks.

An environmental DNA-based study from the USA in wood frogs (L*. sylvatica*) found that the timing of pathogen introduction affected the timing of epidemics or the resulting die-offs that appears to be driven by development- and/or temperature-dependent changes in pathogenicity (Hall *et al.,* 2018). Outbreaks of Epizootic Haematopoietic Necrosis (EHNV) infecting redfin perch (*Perca fluviatilis*) in north‐eastern Victoria, Australia, was recorded in November-December (early summer in the Southern Hemisphere) (Langdon and Humphrey, 1987). Incidence of Largemouth bass virus (LMBV) die-offs in summer was driven by the increased susceptibility to infection of these fish at higher temperatures (Grizzle and Brunner, 2003). However, there is evidence that when ranavirus epidemics occur during late spring or summer, they are of shorter duration—only a few weeks (Green *et al.,* 2002). Broadly, all these studies indicate greater disease incidence in the summer months.

Only a few studies report seasonality in ranavirus outbreaks in Asia. Few outbreaks have been recorded in Asian amphibians during summer, most of these in cultured species, and patterns of seasonality of infection in Asia remain to be discerned. In many instances, the disease is reported only when mass mortalities occur. One study recorded a mass die-off of exotic North American bullfrogs (*Lithobates catesbeianus,* formerly *Rana catesbeiana*) larvae in Japan from early September to mid-October (Une *et al.,* 2009). Outbreaks have also been reported in cultured Chinese giant salamanders (*Andrias davidianus*) between February and November (Chen *et al.,* 2013; Cunningham *et al.,* 2016; Du *et al.,* 2016; Geng *et al.,* 2011). Further, cultured, black-spotted pond frogs (*Rana nigromaculata*) became infected with *Rana nigromaculata* ranavirus (RNRV) in March, while infections in the cultured Chinese tiger frog (*Hoplobatrachus* cf*. rugulosus*, formerly *R. tigrina rugulosa)* occurred between May and June (Mu *et al.,* 2018; Weng *et al.,* 2002). However, generalizing seasonality of ranaviruses in Asia based on a few studies from a limited number of locations seems inadequate as there may be significant geographic variation. Therefore, it is essential to investigate how ranavirus dynamics vary across latitudes and variability in seasonality.

**Ranavirus infections and climatic change**

Most die-off events caused by ranaviruses begin (and often end) during the summer months (Brunner *et al.,* 2015), which reiterates their affinity with higher temperatures. Temperature can influence both the kinetics of host-parasite interaction and act as a host stressor (Altizer *et al.,* 2013). However, increasing temperatures may not always be stressful to some hosts, depending on the thermal tolerance physiology of the species. Experimental studies show temperature dependent replications by different strains of ranaviruses. Under laboratory conditions, short-finned eel ranavirus (SERV) replicated optimally at 20 °C (Ariel and Jensen, 2009), while LMBV grew slightly faster at 30 °C than at 25 °C (Grant *et al.,* 2003). Higher mortality rates are in many cases associated with higher temperatures. Redfin perch and rainbow trout infected with EHNV show increased mortality (Ariel and Jensen, 2009). Higher mortality was observed when common frog tadpoles were exposed to ranaviruses at higher temperature (Gray *et al*., 2009). Although there are a few exceptions (Rojas *et al.,* 2005; Allender *et al.,* 2018), most studies suggest that higher temperatures stress host species infected with ranviruses (Ariel and Jensen, 2009; Bayley *et al.,* 2013; Echaubard *et al.,* 2014; Price *et al.,* 2019).

Global warming too, is set to affect the epidemiology of ranaviruses. Climate projections suggest that climate change will likely play a significant role in shaping future ranavirus disease dynamics in amphibians, altering both the geographic extent and the length of the temporal window for disease risk and severity (Price *et al.,* 2019). Furthermore, unprecedented urbanization in Asia, together with associated temperature increases, (Song *et al.,* 2003, Wang *et al*., 2014) may exacerbate ranaviral outbreaks in the region.

**Diagnostic tests**

Several methods are used to detect ranaviruses. Earlier methods included electron microscopy (EM), histopathology and cytology. Newer approaches include virus isolation, antigen-capture enzyme-linked immunosorbent assay (Ag-capture ELISA), immunohistochemistry (IHC), and PCR (Miller *et al.,* 2015).In both conventional and real-time PCR (qPCR), the major capsid protein (MCP) gene, neurofilament triplet H1 protein, DNA polymerase, and an intergenic variable region can be used as targets (Mao *et al.,* 1997; Hyatt *et al.,* 2000; Holopainen *et al.,* 2009; Jancovich *et al.,* 2005). PCR is currently the most widely used and accepted approach for detection of ranaviruses. The highly conserved MCP gene is widely used in ranavirus detection. Dearth of PCR machines and operating costs remain a challenge in some parts of Asia, and are contributing factors for the fewer studies in the region.

**Ranavirus infections in Asia**

Ranaviral infections have been recorded in China, Taiwan, Japan, South Korea, Thailand, Singapore, Malaysia Cambodia, Vietnam and India (Chen *et al.,* 1999; Chen *et al.*, 2013; Cunningham *et al.,* 2016; Deng *et al.,* 2011; Deng *et al.,* 2020; Du *et al.,* 2016; Fu *et al.,* 2017; George *et al.,* 2015; Hazeri *et al.,* 2016; Hazeri *et al.*, 2017; He *et al.,* 2002; Huang *et al.,* 2009; Geng *et al.,* 2011; Kayansamruaj *et al.,* 2017; Kwon *et al.,* 2017; Lai *et al.,* 2000; Mu *et al.,* 2018; Murali *et al.,* 2002; Park *et al.,* 2021; Qi Zhu *et al.,* 2016; Qin *et al.,* 2003; Tamukai *et al.,* 2016; Sivasankar *et al.,* 2017; Sriwanayos *et al.,* 2020; Une *et al.,* 2009; Weng *et al.,* 2002; Xu *et al.,* 2010;Yu *et al.,* 2015; Yuan *et al.,* 2016; Zhang *et al.,* 2001) (Fig. 1). Infections have also been recorded from amphibians traded via Hong Kong (Kolby *et al.,* 2014). It seems that other countries lack surveillance or detection data, which obscures understanding the true disease burden in the region.

The first case of ranavirus infection in Asia was reported in the cultured pig frog (*L. grylio*), in China (Zhang *et al.,* 1996; Zhang *et al.,* 2001). Preliminary studies on virus isolation and cell infection from diseased *L. grylio* were published by Zhang *et al.*, (1996), while characterization of the virus by histopathology, electron microscopy, serological reactivity, gel electrophoresis of viral polypeptides and DNA restriction fragments, PCR amplification, and nucleic acid sequence analysis of the (MCP) gene followed (Zhang *et al*., 2001). Only a few countries carry out surveillance, and the preponderance of monitoring has been confined to species that are cultured either for food or the pet industry. The limited intensity of surveillance implies a high probability of ranaviruses infecting wild species undetected (Park *et al.,* 2021; Kwon *et al.,* 2017; Qi Zhu *et al.,* 2016)

In Asia, ranavirus infections have been recorded in all ectothermic vertebrate groups (fish, amphibians, and reptiles), with the greatest proportion of cases recorded in China. *Rana nigromaculata* ranavirus (RNRV; similar to FV3), Tiger frog virus (TFV), RGV (*Rana grylio* virus), Chinese giant salamander virus (CGSV), Chinese giant salamander (*Andrias davidianus*) iridovirus (CGSIV), *Andrias davidianus* ranavirus (ADRV), RCV-JP (Similar to *Rana catesbeiana* virus), Asian grass frog ranavirus (AGFRV), and East Asian bullfrog Ranavirus (EABRV) are the ranaviral species (isolates) or proposed species infecting amphibians in Asia.

Fish-infecting ranaviral species (or isolates) or proposed species are known to be: Santee-Cooper ranavirus, Triplophysa siluroides ranavirus (similar to Common Midwife Toad Virus (CMTV)-like ranavirus clade), Grouper iridovirus (GIV), Singapore grouper iridovirus (SGIV), Koi ranavirus (KIRV), Similar damselfish virus (SRDV; similar to largemouth bass virus), Oxyeleotris marmorata ranavirus (OMRV), Poecilia reticulata ranavirus (PPRV), and Goldfish ranavirus (GFRV).

Soft-shelled turtle iridovirus (STIV) and Inland bearded dragon ranavirus (IBDRV) are the only two known ranaviral isolates infecting reptiles. There are at least seven other species recorded in the region, but specific names have not yet been given to these. So far, the region harbors four out of seven ranavirus species (or ranavirus isolates considered members of the same viral species) recognized by the International Committee on Taxonomy of Viruses (Fig. 2). *Ambystoma tigrinum* virus, *Epizootic haematopoietic* *necrosis virus* and *European North Atlantic ranavirus* (or their isolates) are the only two species that have not been reported from the region.

**Mainland China**

Ranavirus infections have been recorded in all ectothermic vertebrate groups in China. The screening efforts remain modest considering the risks from intensive aquaculture, ranaculture and mariculture, as well as the pet industry (Fig. 1). These viruses have caused alarming wild-population declines in species of conservation importance such as the Chinese giant salamanders (*A. davidianus*) and native anuran species such as *Rana dybowskii* and *R. amurensi*. Further, ranaviruses have caused diseases in fish species of economic importance such as the largemouth bass (*Micropterus* *salmoides*), catfish‐like loach (*Triplophysa siluorides*), Chinese perch (*Siniperca chuatsi*) and snakehead fish (*Channa maculata*), in addition to pig frogs (*R. grylio*), black-spotted pond frogs (*R. nigromaculata*), Chinese edible frog (*Hoplobatrachus rugulosus),* soft-shelled turtle *(Trionyx sinensis)* andalligator snapping turtles *(Macrochelys temminckii).*

A study of wild dybowski's frogs (*R. dybowskii*) in Heilongjiang Province shows that the overall infection prevalence is 5.7% in adults and 42.5% in tadpoles (Xu *et al.,* 2010). Overall prevalence of infection in adults was shown to be low compared to tadpoles; neither showed clinical signs. Another study highlighted ten strains of FV3-like ranavirus in wild, native *R. dybowskii* and *R. amurensis* in northeastern China (Qi Zhu *et al.,* 2016). Eight of these strains were isolated from *R. dybowskii* from Heihe, Hebei and Dongfanghong, while the remaining two were detected in *R. amurensis* from Hebei. These 10 strains were homologous to FV3, though genetic differences were noted among the isolates.

The first report of ranavirus-driven mass mortality in Chinese giant salamanders (*A. davidianus*) was recorded among farmed populations in Shanxi Province (Geng *et al.,* 2011), where all specimens collected tested positive for ranavirus. The Chinese giant salamander is one of the largest amphibians in the world and it is Critically Endangered (IUCN Red List, 2012) because of threats such as habitat loss and over-harvesting. Emerging infectious diseases such as the Chinese giant salamander iridovirus (CGSIV) can also cause population declines, with mass mortality events increasing their extinction risk (Dong *et al.,* 2011; Meng *et al.,* 2014; Wang *et al.,* 2014). Adult Chinese giant salamanders have been shown to be infected in natural habitats and/or farms in Hunan, Jiangxi and Henan Provinces during 2011-2012 (Chen *et al*., 2013). The isolate responsible for this infection was provisionally designated as *Andrias davidianus* ranavirus (ADRV). It is evident that some Chinese giant salamanders with low levels of CGSIV survive infection. A study shows that autophagy may play a role in survival during the early stages of infection and subsequent proliferation (Du *et al.,* 2016).

Frog Virus 3- (FV3)-like iridoviruses can cause widespread, severe disease and mortality among cultured pig frogs (*L. grylio*), and farms in Hubei Province and Hunan Province have experienced mortality rates above 90% (Zhang *et al.,* 2001). Another outbreak was recorded in cultured Chinese edible frogs (*Hoplobatrachus rugulosus*)in culture facilities in Guangdong and Hainan Provinces, which was also closely related to FV3 (Weng *et al.,* 2002). The infection was prevalent in May to June, and mortality rates of about 95% were observed in tadpoles. Tiger frog virus in a captive population of *H. rugulosus* in Nanhai, Guangdong in Southern China, caused high mortality of tadpoles (He *et al.,* 2002, Yuan *et al.,* 2016). Further, tadpoles of a cultured population of black-spotted pond frogs (*R. nigromaculata*) in Shuangliu County, China, were diagnosed with a FV3-like ranavirus infection where substantial mortality was reported (90%; Mu *et al.,* 2018).

High mortality in cultured largemouth bass (*Micropterus salmoides*) was observed in Foshan, Guangdong Province in 2008 and the virus was identified as identical to doctor fish virus (DFV), a pathogen closely related to largemouth bass virus (LMBV) (Deng *et al.,* 2011). Another high-mortality event occurred due to Common Midwife Toad Virus (CMTV)‐like ranavirus in a cultured loach (*Triplophysa siluorides*) in Sichuan Province, which affected about 75% of the stock (Deng *et al.,* 2020). A Chinese giant salamander farm, with cases of CGSV infection, was located approximately 1 km upstream of the *T.* *siluroides* farm. It was suspected that the virus was transmitted by contaminated water from the former.

Isolates of Santee-Cooper ranaviruses from diseased Chinese perch (*Siniperca chuatsi*) and snakehead fish (*Channa maculata*) from Guandong Province provide further evidence that infection may be widespread in China (Fu *et al.,* 2017).

Soft-shelled turtle iridovirus (STIV) responsible for the novel viral disease called 'red neck disease' in the farmed soft-shelled turtle (*Trionyx sinensis*) have been recorded in farms in Shenzhen (Chen *at al.,* 1999). Phylogenetic analyses suggested that STIV and FV3 are closely related and may transmit between reptiles and amphibians (Huang *et al.,* 2009). Another case of ranavirus in testudines was recorded in snapping turtles (*Macrochelys temminckii*) collected from an aquarium in Sichuan Province, showing symptoms such as local inflammation and swelling in the neck and limbs, along with ulcerated and perforated plastrons (Yu *et al.,* 2015).

**Hong Kong SAR, China**

Kolby *et al*., (2014) conducted a surveillance project to investigate the presence of ranaviruses and chytrid fungus (*Batrachochytrium* *dendrobatidis*) in commercial shipments of live amphibians exported from Hong Kong to USA. Sampling upon arrival in USA indicated that ranavirus was present in 105/185 (56.8%) of the exported amphibian specimens. However, it remains unclear how the native amphibians in Hong Kong contracted the virus.

**Taiwan, China**

Grouper iridovirus (GIV) was isolated from yellow groupers (*Epinephelus awoara*) in a ﬁsh farm in Hsiau Liouchiou Island (Lai *et al.* 2000; Murali *et al.,* 2002), where mortality reached 100% within 11 to 25 days post-infection (Murali *et al*., 2002). Futhermore, twenty-three iridovirus isolates from both seawater and freshwater fish from farms distributed throughout all seven Taiwan prefectures (Pingtung, Kaohsiung, Penghu, Tainan, Chiayi, Nantou and Taipei) were recorded between 2001 and 2009 by Huang *et al.* (2011). The extracts were collected from eight species of cultured fish: 11 isolates from giant grouper (*Epinephelus lanceolatus*), five from orange-spotted grouper (*E. coioides*), three from giant seaperch (*Lates calcarifer*), one from crimson snapper (*Lutjanus erythropterus*), one from silver sea bream (*Rhabdosargus sarba*), one from largemouth bass (*M. salmoides*), one from rock bream (*Oplegnathus fasciatus*), and one from marble goby (*Oxyeleotris marmoratus*). The 23 isolates were divided into six groups within the genera *Ranavirus* and *Megalocytivirus*. Prior to 2005, the viruses were closely related to members of the genus *Ranavirus,* while after 2005, they were similar to members of the genus *Megalocytivirus*. In *Ranavirus*, the bootstrap values supported three major groups: three isolates were closely related to the GIV (group V), eight were related to the SGIV (group VI) and FV3, while TFV was in group IV. The phylogenetic analysis of viral genomic DNA in the MCP genes showed that the genotypes of these isolates were closely related to SGIV and GIV. Given the wide range of habitats and high number of potential host species (76 reptiles, 30 amphibians and 130 freshwater fishes) present in Taiwan (WWF, 2020), the propensity for the pathogen to spread should be closely monitored.

**Japan**

Biogeographically isolated from the rest of Asia by the Sea of Japan, Japan harbors a high amphibian diversity. There is also a high risk of disease-spread from imported aquatic organisms . Wild North American bullfrog (L. catesbeiana) larvae suffered a mass die off in a pond in western Japan during the autumn of 2008, attributed to RCV-JP (Une *et al.,* 2009). Lethargy, palpebral hyperemia, abdominal edema, petechiae, and erythema on the ventral surface, skin ulcers, limb and tail necrosis and emaciation were among the common clinical signs.

Another ranavirus outbreak was detected in a population of inland bearded dragons (*Pogona vitticeps*; n=100) at a breeding facility in Japan. This was named the inland bearded-dragon ranavirus (IBDRV) and MCP gene sequence analysis showed it to be similar to the three ranaviruses described in infected amphibians in Japan, Korea and Taiwan. Reptilian ranaviruses, which often cluster closely with amphibian ranaviruses (FV3-like, TFV-like or CMTV-like), were also detected from the vicinity of the breeding facility, from which horizontal transmission may have occurred (Tamukai *et al.,* 2016).

**South Korea**

There are two cases of ranaviral infections reported from South Korea. The first of these was a mass-mortality event in a natural population of huanren frog (*R. huanrenensis*) tadpoles by a ranavius closely related to the *Rana catesbeiana* virus JP MCP, isolated from invasive bullfrog tadpoles in Japan (Kwon *et al.,* 2017). This study failed to detect any lethal bacteria or chytrid fungus on the specimens; this is perhaps the best known case of ranavirosis implicated in a mass mortality event of an endemic wild amphibian in Asia.

Another mass mortality event involving an adult population of dybowski’s brown frogs (*R. dybowskii*) was detected in 2017, from a stream in Moksang-dongin (Park *et al.,* 2021). The MCP sequence resembled the Frog virus 3 (FV3) that had been collected earlier from huanren brown frog (*R. huanrenensis*) tadpoles in South Korea.

**Thailand**

Several ranaviruses were isolated from Thailand’s aquaculture facilities between 1998 and 2001 (Sriwanayos *et al.,* 2020). These were identified in marbled sleeper goby (*Oxyeleotris marmorata*), goldfish (*Carassius auratus*), guppy (*Poecilia reticulata*), tiger frog (*H. tigerinus*), Asian grass frog (*Fejervarya limnocharis*), and East Asian bullfrog (*H. rugulosus*). Asian grass frogs (*F. limnocharis)* had been imported from Cambodia, while the other species were cultured in situ. Both adults and tadpoles of East Asian bullfrogs had shown elevated mortalities with cutaneous ulcerations. Phylogenomic analyses implicated eight Thai ranaviruses, which showed similarity to Chinese Tiger Frog Virus (TFV) and Wamena virus (WV), as a subclade within a larger frog virus 3 clade. Opportunities for interclass transmission of TFVs are possible at large pet markets, where tadpoles and frogs are housed in open containers next to ornamental fishes, with risk of water being exchanged. Further, feeding of juvenile frogs to large predatory ornamental fishes may also have contributed to the spread of TFVs in Thailand.

Another ranavirus infection associated with an outbreak of ulcerative disease among barcoo grunter fish (*Scortum barcoo*) in farms in the central region of Thailand (Ayutthaya and Phetchaburi provinces) was detected in 2013 (Kayansamruaj *et al.,* 2017). These freshwater fish, a popular aquaculture species, are native to Australia and had recently been introduced to Thailand.

**Singapore**

A newly isolated grouper virus from a diseased brown-spotted grouper (*Epinephelus tauvina*), related to largemouth bass virus (LMBV), FV3 and Regina ranavirus (RRV), was named as Singapore grouper iridovirus (SGIV; Qin *et al.,* 2003). SGIV was shown to cause serious systemic disease capable of killing 96% of grouper fry. Mariculture of *Epinephelus* species is rapidly developing in Singapore and other Southeast Asian countries, to which this virus poses a serious threat.

Outbreaks of a novel viral disease called ‘Sleepy Grouper Disease’ (SGD) was first observed in *E. tauvina* in Singapore in 1994 (electron microscopic analyses), causing economic losses (Chua *et al.* 1994). However, the virus strain was not verified at the time by cell culture techniques. Later, it was identified as SGIV. Another outbreak of the same disease occurred in fry and adults of brown-spotted groupers in 1998. These fry were imported from other Southeast Asian countries and the outbreak lasted several weeks, resulting in more than 90% mortality.

**India**

There are two instances of ranavirus infections recorded from India. The first was in freshwater fishes, following a mass mortality event. A virus resembling Santee-Cooper Ranavirus (MCP gene showed a 99.9% similarity to that of largemouth bass virus isolated from North America) from koi carp (*Cyprinus rubrofuscus*) in ornamental fish farms of South India (George *et al.,* 2015). It was a rigorous study based on virus isolation, electron microscopy and PCR-based detection, followed by sequencing of capsid protein gene and transmission studies. Darkening of skin, loss of scales, vertical orientation, uncoordinated and inverted swimming, lateral rotation, intermittent surfacing, and settling at the bottom laterally were the commonly observed clinical signs.

The second case was reported from farm-reared similar damselfish (*Pomacentrus similis*) with frequent mortality events reported from marine ornamental fish farms of South India (Sivasankar *et al*., 2017). The name ‘Similar damselfish virus’ (SRDV) was proposed and the MCP gene showed a close relationship to largemouth bass virus (LMBV).

**Cambodia and Vietnam**

The only study conducted so far in Cambodia and Vietnam failed to detect ranavirus in these countries (Gilbert *et al.,* 2012). The screening was based on qPCR and histopathology of liver and other tissue samples collected from 74 frogs, with most samples being from Cambodia (n = 70).

**The Philippines**

A study carried out to investigate ranaviruses along with *Batrachochytrium dendrobatidis* among wild amphibians (n = 304, from seven sites) from the Philippine islands of Luzon, Negros, Calayan and Camiguin Norte failed to detect ranaviruses (Smith *et al.,* 2019).

**Malaysia**

Groupers are the only group in which ranaviruses have been detected in Malaysia so far. Grouper iridovirus (GIV), which shows close phylogenetic relationship to other grouper iridoviruses from Asia, was recorded in both tiger grouper hybrid (*Epinephelus* sp.) and coral trout (*Plectropomus leopardus*;Hazeri *et al.,* 2016; Hazeri *et al.,* 2017). Groupers are popular aquaculture fish species in Malaysia that are frequently affected by disease outbreaks.

**Severity of ranavirus infections at global scale**

Some host species are highly susceptible to ranaviruses. Experimental evidence suggests that the effects of novel strains of ranaviruses introduced into native populations could have devastating consequences (Duffus *et al.,* 2015). Pathogen surveillance and viral dynamics using full genomes can be used to better understand the mechanisms of disease origin and spread along with commercial trade. Recombination events have occurred between CMTV-like ranaviruses and FV3-like viruses that are isolated from the wild strains in Canada (Vilaca *et al.,* 2019). Both these viruses may have spread to North America when the international commercial amphibian trade started. These different recombination patterns in the FV3-like ranavirus can increase the risk of causing the disease (Vilaca *et al.,* 2019). Despite limited monitoring efforts, the alarming increase in recent reports of ranavirus emergence in Asia may be an underlying reason for unexplained population declines such as in the case of Chinese giant salamanders (Dong *et al*., 2011; Meng *et al.,* 2014; Wang *et al.,* 2014). High mortality rates of hosts, diverse host range (and hence the potential to affect numerous novel species) has prompted the World Organization for Animal Health (OIE) to list ranavirus as a notifiable disease (i.e., transmissible diseases that have the potential for profoundly serious and rapid spread, irrespective of national borders, that entail serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products). *Epizootic haematopoietic necrosis virus* is listed as a fish disease, while infection by ranavirus species is listed as amphibian disease in the OIE’s listed diseases (World Organization for Animal Health, 2021). This designation requires countries (which have subscribed to OIE policies) to screen a sample of ranavirus hosts that cross international borders for ranaviruses (Schloegel *et al.,* 2010). Quarterly Aquatic Animal Disease Report (Asia-Pacific Region) includes data on disease prevalence of ranaviruses because the disease has become prevalent in the region (Network of Aquaculture Centers in Asia-Pacific and Food, World Organization for Animal Health (OIE) Regional Representation for Asia and the Pacific and Agriculture Organization of the United Nations, 2020),

**Introducing ranaviruses to** **uninfected areas and transmission within Asia**

Growing international trade of live amphibians, reptiles and fish, taken from the wild or bred in captivity, and sold commercially as food or ornamental species/pets, appears to increase the risk of introducing and dispersing ranaviruses across Asia. Several studies suggest that many species imported to Asia could potentially host ranaviruses (Table 1).

It is possible that ranavirus-caused diseases may have existed undetected in Asia over an extended period, though now coming to be better understood as a result of the widespread application of molecular diagnosis techniques. Alternatively, the detected outbreaks could signal a recent emerging infection spreading rapidly across the world because of the ever-increasing mobility of pathogens due to global trade of live animals.

Phylogenomic analyses can explain disease dynamics. For instance, putative recombinants between FV3, a pathogen widely distributed within wild populations, and CMTV, have caused high pathogenicity. While CMTV-derived genes associated with virulence are reported in wild strains in Canada, FV3 has been linked to amphibian die-offs in North America (Vilaca *et al*., 2019). The latter study provides an insight on how pathogen surveillance and viral dynamics using full genomes can be used to understand the mechanisms of disease origin and spread more clearly.

It is a possible that ranaviruses can be transmitted between ectothermic vertebrate classes through water (Brenes *et al.,* 2014). Further, fish and reptiles might serve as reservoirs for ranavirus, given their ability to live with subclinical infections, which may contribute to the pathogen’s persistence, especially when highly susceptible hosts like amphibians are absent due seasonal population fluctuations.

Studies from Asia indicate that ranavirus infections cross species barriers, allowing the virus to infect previously uninfected indigenous hosts. Further, given the high mutability of ranaviruses, new strains can emerge (Chen *et al.,* 2013).

Some of the introduced host species are known to be infected with ranaviruses in their original locations. One of the best examples is in China, where several imported species that can harbor the infection have been introduced. The Pig frog (*R. grylio*) and American Bullfrog (*L. catesbeianus*) are popular cultured species introduced from the United States to China. These have been bred and distributed throughout China (Qi Zhu *et al.,* 2016; Zhang, 2001). Largemouth bass (*M. salmoides*), which were identified as being infected with a ranavirus identical to doctor fish virus (DFV) or a strain of DFV, has also been imported to China from USA (Deng *et al.,* 2011). Largemouth bass virus (LMBV), closely related to DFV, has been recorded in largemouth bass in South Carolina's Santee-Cooper reservoir, though the origin of the virus has not been confirmed (Mao *et al.,* 1999b). Additionally, exotic species being imported as pets, such as red-eared sliders (*T. scripta elegans*) and snapping turtles (*M. temminckii*) are known to harbor ranaviruses and are widely traded in China (Yu *et al.,* 2015; Moore *et al.,* 2014).

There are a few records from other countries in the region as well. The barcoo grunter fish (*S. barcoo*), which is imported from Australia and cultured in Thailand, was infected with a ranavirus similar to Largemouth bass virus (LMBV) (Kayansamruaj *et al.,* 2017). Inland bearded dragons (*P. vitticeps*), a pet species imported to Japan, was found to be infected with Inland bearded dragon ranavirus (IBDRV), though the details of origin are not available (Tamukai *et al.,* 2016). Meanwhile, Koiranavirus (KIRV) has been recorded among imported koi carp (*C.* *rubrofuscus*) in India (George *et al.,* 2015).

In addition, some of the ranavirus infections may already have been transmitted among Asian countries. Asian grass frog ranavirus (AGFRV) has been recorded in Asian grass frogs (*F. limnocharis*) imported from Cambodia to Thailand’s culture facilities (Sriwanayos *et al.,* 2020). Meanwhile, grouper fry imported from other Southeast Asian countries might have carried the Singapore grouper iridovirus (SGIV) into Singapore and Malaysia (Hazeri *et al.,* 2016: Hazeri *et al.,*2017; Qin *et al.,* 2003).

**Potential entry of ranavirus to Asia through cultured frogs -** **American bullfrog (*Lithobates catesbeianus*) and *R. grylio* as reservoirs**

One of the species highlighted so far in this regard is the American bullfrog (*L. catesbeianus*), which seems to have played a key role in spreading the pathogen to new locations (Both *et al*., 2011; Mazzoni *et al.,* 2009: Ruggeri *et al.,* 2019; Schloegel *et al.,* 2009; Schloegel *et al.,* 2010). Cultured American bullfrogs often carry ranavirus infection FV3 (Miller *et al.,* 2007) and may have served as a vector transmitting the disease to native amphibians and fish in Brazil (Mazzoni *et al.,* 2009; Ruggeri *et al.,* 2019). It is striking that American bullfrogs have been introduced and now occur in nearly 40 countries in Africa, Asia, and North, Central, and South America, and islands of the Mediterranean, South Pacific and Caribbean (Kraus, 2009). These frogs are of particular concern as vectors of the disease as they are capable of being infected without showing clinical symptoms typical of ranavirosis (Hoverman *et al.,* 2011). Bull frog ranaculture may have facilitated recombinations of different species of ranaviruses with enhanced pathogenicity. A chimeric ranavirus that displayed a novel genome arrangement between FV3 and CMTV was observed in a North American farm by Claytor *et al.,* (2017). Further, there is increased risk of new strains, which are different from existing ones, emerging (Oliveira *et al.*, 2020). Thus, the international trade in farmed bullfrogs may have contributed to dispersal of highly pathogenic ranaviruses globally.

There are accounts of North American bullfrogs being introduced from Japan (by the Shanghai Fisheries University) to China (Ningbo and Tianjin cities and Guangdong province) between 1958 to 1961 for breeding and distribution (Qi Zhu *et al.,* 2016). Viral isolates from Southern China were similar to the North American bullfrog isolates from Japan, which indicates the long-term trade exchange. Interestingly, a ranavirus named RCV-JP has been identified in cultured North American Bullfrogs in Japan (Une *et al.,* 2009). This species is similar to rana catesbeianavirus identified from North American bullfrogs in a frog farm in the USA (Majji *et al.,* 2006), which suggests that the infection may have persisted in these frogs when they were introduced to Japan from the USA.

Further, *R. grylio*, which is native to the south-eastern United States, has been imported into China for farming purposes. There is a possibility that these imported frogs harbored the virus and were the focus of the initial infection (Zhang, 2001). The evidence of recording the RGV *(Rana grylio* virus) which is similar to the FV3 virus among cultured pig frogs (*L. grylio*)inChina could provide more evidence of introduction of the disease from USA, as this species too, had been imported.

**Potential transmission to native/endemic species: jumping species barriers**

Ranavirus infections that have been recorded in Chinese Giant salamander may have been transmitted from pig frogs(*L. grylio)* that are routinely fed to farmed Chinese Giant Salamanders (Cunningham *et al.,* 2016). This is supported by the fact that ranavirus from Chinese giant salamanders in Sichuan Province show a close relationship to ranavirus in pig frogs (Cunningham *et al.,* 2016; Zhou *et al.,* 2013). This hypothesis is further supported by ADRV having been shown to be more closely related to frog (anuran)-infecting ranaviruses such as CMTV, RGV FV3 and TFV than ATV, which is the salamander (urodele)-infecting ranavirus (Chen *et al.,* 2013). The phylogeny we constructed from existing data supports this hypothesis (Fig. 2). Further, Triplophysa siluroides ranavirus infecting cultured catfish-like loach (*Triplophysa siluorides*) in China could potentially have originated in a Chinese giant salamander farm, in which water may have been the vector for the CGSV infection (the salamander farm is located approximately 1 km upstream of the loach farm: Deng *et al.,* 2020).

Potential interclass transmission of TFVs in Thailand may have occurred in live animal markets where tadpoles and frogs are housed in open containers next to ornamental fishes, in addition to the feeding of juvenile frogs to large predatory ornamental fishes (Sriwanayos *et al.,* 2020).

**Biosecurity and preventing further entry of ranaviruses to Asia**

Import risk analysis (IRA) is a procedure used to determine the threat of a pathogen entering a system with international trade in animals and their products. This has been largely driven by the Sanitary and Phytosanitary (SPS) agreement of the World Trade Organization (WTO) and the IRA standard established by the World Organization for Animal Health (OIE), which provides an IRA standard aquatic and terrestrial animal health codes (O.I.E., 2019; Peeler *et al.,* 2013). The principal aim of import risk analysis is to provide importing countries with an objective and defensible method of assessing the disease risks associated with the importation of animals and their products. The risk assessment is the component of the analysis that estimates the risks associated with a hazard. Risk assessment is composed of entry assessment, exposure assessment, consequence assessment and risk estimation. Risk management deals with deciding upon and implementing measures to address the risks identified in the assessment. This will ensure that negative effects on trade are minimized.

IRAs can also be used to establish or revise trade or translocation guidelines for wildlife that could be subclinically infected with a pathogen (Smith *et al.,* 2009). Risk analysis on wildlife species in trade, pre-border pathogen screening, and voluntary support should help reduce costs associated with species invasion as well as protecting the public, and enhancing environmental and animal health (Smith *et al.,* 2009). Pandora+ is a protocol that assesses the risk of a particular pathogen or parasite being introduced by a particular host species (D’hondt *et al.,* 2015). Harmonia+ is used to distinguish the components of invasion (D’hondt *et al*., 2015). Pandora+, along with Harmonia+, can successfully be used to assess the risk of pathogens in invasive species. The Pandora+ protocol has been successfully used to demonstrate a high risk for pathogens with potential to affect amphibians from neighboring regions in Sinaloa, Mexico, following the first report of a ranavirus outbreak in farmed American Bullfrogs (*L. catesbeianus*; Saucedo *et al.,* 2019). These techniques can successfully be used also to assess risk in Asia and prevent further introductions of high-risk host species to the region. Further, it is important to carry out disease surveillance for all ectothermic species now being cultured to prevent further disease spread to wild species.

When a ranavirus case is reported it is important to report it to responsible government agencies and to the World Organisation for Animal Health (OIE) through relevant channels. There is an urgent need for improved biosecurity practices and a better understanding of the pathogen-host-environment imbalance often created under artificial culture conditions (Chinchar and Waltzek, 2014) in both aquaculture/ranaculture and the pet trade in Asia, especially given that the existing measures are clearly inadequate. Disease prevention, driven by legislation and effective regulation, is fundamental to the sustainability of the aquaculture industry (Gudding *et al.,* 2012). Meanwhile, existing national, regional as well as international laws and regulations can be successfully used to improve biosecurity measures related to aquaculture practices for minimizing the risk of disease emergence and transmission.

Identification of national priorities for aquatic animal health management and the development of national strategies are important to all the countries of Asia-Pacific (Aus Aid and Network of Aquaculture Centres, 2006). Since ranaviruses pose a high risk to Asia, each country in the region should be equipped to conduct import risk analysis. The authorities dealing with animal health should screen imported consignments of live ectothermic animals and their products for pathogens according to established risk assessment criteria before allowing them in. The records of presence of ranaviruses should be shared transparently in the region as collective effort is important in controlling the disease. Developing such a framework for surveillance and reporting, as well as a framework for contingency planning in Asia, are vital steps in controlling infections in the region. Many countries, meanwhile, lack infrastructure and expertise when it comes to disease diagnosis, surveillance, quarantine, and risk analysis needed to control infections in the region. Therefore, an effective legal framework, along with enhanced disease surveillance and biosecurity measures, is required to control potential pathogen introductions as well as to minimize risk of disease transmission.

**Conclusions and future directions**

Ranaviral infections have up to now been recorded in at least twelve countries and/or administrative regions of Asia. Our review indicates that the surveillance effort in Asia is inadequate. China has lead surveillance and research in the region. However, most of the surveillance work carried out in the rest of Asia has been sporadic and opportunistic, and confined to species of economic value given that investigations have focused on major outbreaks and die-offs. Some countries with high potential host diversity, such as Laos, Myanmar, Brunei, Bangladesh, Bhutan, Nepal, Pakistan, and Sri Lanka lack any records of surveillance work. There is an urgent need to establish the possible presence of ranaviruses in these countries. Infections have been recorded in all the three ectothermic vertebrate classes both in cultured species (indigenous/endemic or introduced) as well as in wild species (Table 1/Fig. 2). Infections spread rapidly in cultured populations with low genetic variability. A large number of animals belonging to different classes as well as species are stocked in small spaces, causing stress in animals, while feeding diseased animals to other species can facilitate spread of infection. Introduction of American bullfrogs (*L. catesbeianus*) and Pig frogs (*L. grylio*) for ranaculture, largemouth bass (*M. salmoides*) for aquaculture and pet species such as Red-eared sliders (*T. scripta elegans*), which are known to carry ranaviral infections in their original home ranges, appear to exacerbate the dispersal of infection in Asia. The growing popular industry of Grouper culture could potentially spread Grouper iridovirus (GIV) in the whole region. Further, infected animals may be deliberately or accidently released to the wild, while infection could also be transmitted via other media such as contaminated waste. Further, it seems that immune evasion strategies of ranaviruses that are developed during virus-host co-evolution, serve to make new strains of the virus more virulent. Given that ranaviruses are often reported during summer months, rising temperatures associated with climate change may facilitate ranavirus spread. Mass die-offs have been recorded in several countries such as China and Japan. So far, no effective treatment to reduce mortality or morbidity has been found. Therefore, control measures, such as limiting international trade of animals and screening for disease, must be strictly followed. Molecular diagnostic techniques can be successfully used to observe the phylogenetic relationships and their host ranges, to detect the possible original sources of introduction. Well-planned, widely distributed systematic screening is a must to understand the prevalence and impact in the Asian region, to conserve the biodiversity as well as to safeguard the widely established and economically important aquaculture industry.

**Figure 1. Regional distribution of Asian ranavirus cases by country**

Hosts of ranaviruses (salamanders, anurans, fish, agamid lizards and testudines) are indicated by the respective silhouettes of hosts.

**Figure 2. Asian ranaviruses on a phylogeny:** Phylogenetic perspective of Asian ranaviruses (highlighted in red) in the context of broad virus type (Frog virus (FV3)-like, common midwife toad virus (CMTV)-like and Ambystoma tigrinum virus (ATV)-like). Hosts of Asian ranaviruses (salamanders, anurans, fish and testudines) are indicated by the respective silhouettes of hosts. The overall topology of the tree was obtained by a two-stage Bayesian approach PASTIS (Thomas *et al.,* 2013) which uses a backbone topology based on molecular data, a set of taxonomic postulates and user-defined priors on branch lengths and topologies to generate a posterior distribution of complete ultrametric trees that capture uncertainty under a homogeneous birth-death prior model of diversification and placement constraints. Twenty-five complete genomes (~100,000 bp) desposited in Genbank (last accessed on February 2021) were aligned in MAFFT and were analyzed in several tree-building programs such as MAFFT, IQtree, NDtree, and CSI phylogeny, using the best fitting model as TVMe+R2, which provided topologies congruent with those of Chinchar *et al.,* (2017). The tree output given by the MAFFT (i.e., the ultrametric tree) was used as the backbone tree in the analysis. To incorporate isolates with short sequence data into the backbone tree, molecular data for several loci (MCP, DPG, RDRASPG and RDRBS) for all taxa included in the tree were downloaded from Genbank. They were aligned in MEGA using MUSCLE, were softly constrained in to their specific broad virus type based on the taxonomic information available in the literature using PASTIS, and were analyzed in Mr.Bayes by running for 60 million generations. As soft constraints were allowed to move freely on branches, they were able to fix at places with maximum likelihood values, revealing possible relationships of taxa whose phylogenetic positions were previously unknown or doubtful. Branches with posterior probability values >95% are marked by asterisks. Lymphocystis disease virus from China was used as the outgroup. World map in the sub-figure shows known locations of ranaviruses, in which the highlighted extent depicts the Asian region considered in this paper. Note: For detailed descriptions of ranavirus isolates in Asia, see Table 1. Note also that Inland bearded dragon ranavirus, Koi ranavirus, Oxyeleotris ranavirus, Goldfish ranavirus, Asian grass frog ranavirus and East Asian bull frog ranavirus are not indicated in the phylogeny as their phylogenetic relationships were unspecified or doubtful.

**Table 1: Regional distribution of Asian ranavirus infections or mortality in wild and captive amphibians**

**Conflict of interests**

None to declare.

**Data accessibility statement**

Data sharing: not applicable. All data and sources used in the study are provided within the framework of the study.

**Author Contribution**

Jayampathi Herath: Conceptualization (lead); writing‐original draft (lead); writing‐review and editing (lead). Gajaba Ellepola: Conceptualization (supporting); writing‐original draft (supporting); writing‐review and editing (supporting), phylogenetic analysis, preparation of the map. Madhava Meegaskumbura: Supervision (lead); conceptualization (equal); writing‐original draft (equal); writing‐review and editing (equal).

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**Table 1: Regional distribution of Asian ranavirus infections or mortality in wild and captive amphibians**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Viral name** **(abbreviation)/**  **Phylogenetic relationship** | **Host Species/**  **life stage** | **Region/Country** | **Wild/**  **Captive/**  **Introduced** | **Month/**  **Year** | **Suspected origin** | **Symptoms** | **Intensity** | **Detection method/****phylogenetic analysis** | **Reference** |
| Rana nigromaculata ranavirus (RNRV)  (FV3-like) | Black-spotted pond frogs (*Rana*  *nigromaculata*) - Tadpoles | Shuangliu  County, China | Cultured | April 2016 | Breeding frogs  bought from Guangdong Province of China.  PCR indicates that the samples of breeding frogs and eggs were negative. But the water samples were positive. River water may contain the virus | Haemorrhage on their body surface, swollen abdomen with yellow ascites,  congestion and swelling of the liver | Approximately 90% of tadpole mortality | Electron microscopy, Challenge experiments  Virus isolation, Electron microscopy  Challenge experiments,,PCR,  MCP gene sequencing and phylogenetic analysis | Mu *et al.,* 2018 |
| \* Not specified  10 strains of FV3-like ranavirus | *Rana dybowskii*  *Rana amurensis* | Heihe, Hebei and Dongfangh, China | Wild | Not available | North American bullfrogs  were introduced from Japan and artificial breeding of frogs begun in mainland  China in the late 1950s  A number of isolates from Southern China were similar to the North American bullfrog isolates from Japan, which may be explained by the long-term trade exchange | Not available | Not available | Viral isolation, Cloning and sequencing | Qi Zhu *et al.,* 2016 |
| \* Not specified  98% homology with Iridovirus RGV | *Rana dybowskii* | Hebei, Dongfanghong, Heihe, Tieli, Huanan, and Hailin regions, China | Wild | Not available | Not available | Not available | Infection prevalence of 5.7% for adults and 42.5% for tadpoles | PCR, MCP gene sequencing and phylogenetic analysis | Xu *et al.,* 2010 |
| Tiger frog virus (TFV) | Tiger frog *(Hoplobatrachus tigerinus,* formerly *Rana tigrina rugulosa) -* Tadpoles | Guangdong, China | Cultured | May to June  2000 | Not available | Abdominal distension, ataxia, reduced feeding | High mortality of tiger frog tadpoles | Viral isolation, Cloning and sequencing, Computer-assisted analyses of the  deduced amino acid sequences | Weng *et al.,* 2002  He *et al.,* 2002, Yuan *et al.,* 2016 |
| Rana grylio virus (RGV)  (Similar to FV3) | Pig frog (*Lithobates grylio,* formerly*, Rana grylio) -* Adults | Wuhan, Hubei province, China | Cultured  Introduced from USA | 1995 - 1998 | Since *Rana grylio* is present in the southeast United States and has been imported into China  for farming purposes, it is possible that imported frogs  harbored the virus and served as the focus for the initial infection | Haemorrhages occurred throughout the leg area. In young frogs, hemorrhagic spots appeared around  neck, back and abdomen, and the skin began to ulcerate | Mortality approached 95% | Virus isolation, electron microscopy, PCR, Nucleic acid sequence analysis | Zhang *et al.*, 2001 |
| Chinese giant salamander virus (CGSV) - Provisional | Chinese giant salamanders  (*Andrias davidianus*) - Adults | Hanzhong County, Shanxi Province, China | Cultured | February to May 2010 | Not available | Anorexia, lethargy,  Ecchymoses, swollen areas on the head, limbs, and skin ulceration | Approximately  350 of a total of 570 salamanders died | Virus isolation, Electron microscopy  ,PCR,  MCP gene sequencing and phylogenetic analysis | Geng *et al.*, 2011 |
| Chinese giant salamander (Andrias davidianus) iridovirus (CGSIV) | Chinese Giant Salamander (*Andrias davidianus*) - Adults | Hanzhong County (Shaanxi Province, China) | Cultured | November, 2014 | Not available | shedding skin | Not available | Histological analysis, Immunohistochemistry (IHC), Immunofluorescence (IF), RT-PCR | Du *et al.,* 2016 |
| Andrias davidianus ranavirus  (ADRV)  More closely related to frog ranaviruses than to other salamander ranaviruses | Chinese Giant Salamander (*Andrias davidianus*)-  Adults, larvae | Hunan, Jiangxi and  Henan Provinces of China | Cultured | May 2011 to August 2012 | ADRV might emerge  from a common ancestor of amphibian-subgroup ranaviruses. Genome characterization and comparison analysis  indicates that ADRV should be a new member of the Amphibian subgroup of ranaviruses genomic architecture changes and several gene variations may contribute to evolutionary emergence of ADRV | Severely hemorrhagic lesions | nearly 100% in  the diseased salamanders | Virus isolation, Electron microscopy  Challenge experiments,  Genome annotation and analysis | Chen *et al.,* 2013 |
| \*Not specified  (Similar to Chinese giant salamander iridovirus  and Rana grylio virus) | Chinese giant salamander (*Andrias davidianu*s) - Adults | Shaanxi Province, China | Cultured | May 210- October 2011 | Possibly feeding Salamanders with pig frogs which is a  North American anuran and introduced to China | Swelling and  bleeding of the head (known locally as big head disease) or  feet (big foot disease), necrosis and bleeding of the oral mucosa  (bad mouth disease) or tail (bad tail disease), and skin  bleeding. | Not available | PCR | Cunningham *et al.,* 2016 |
| Santee-Cooper Ranavirus | Chinese perch (*Siniperca chuatsi*) and snakehead fish (*Channa maculate)* | Guangdong Province, China | Cultured | 2015 | Not available | Ascites, mesentery hemorrhages, pus in the intestine. No specific external signs. | 100% mortality in challenge experiment with *Siniperca chuatsi* | Virus isolation, Electron microscopy  Challenge experiments, PCR,  MCP gene sequencing and phylogenetic analysis | Fu *et al.,* 2017 |
| \*Not specified  (May be identical to doctor fish virus (DFV) or a strain of DFV) | largemouth bass (*Micropterus salmoides*) | Foshan area of Guangdong Province, China | Cultured  Introduced from USA | June to October 2008 | Introduced to China from USA. Might be originated from USA | Ulcerations on the skin and muscle | 100% mortality  (Challenge experiments) | Virus isolation, Electron microscopy  Challenge experiments, PCR,  MCP gene sequencing and phylogenetic analysis | Deng *et al.,* 2011 |
| Triplophysa siluroidesranavirus  (Related to Common Midwife Toad Virus  (CMTV)-like ranavirus clade) | Catfish-like loach (*Triplophysa siluorides)* | Sichuan  Province, China | Cultured | Not available | A Chinese giant salamander farm (with cases of CGSV infection) was located approximately 1 km upstream of the *T. siluroides* farm. Suspected to transmitted by contaminated water from Chinese giant salamander farm | Skin lesions and hemorrhagic  ulcers | 40% to 90% mortality  (Challenge experiments) | Virus isolation, Electron microscopy  Challenge experiments  MCP gene sequencing and phylogenetic analysis | Deng *et al.,* 2020 |
| Soft-shelled turtle iridovirus (STIV) | Soft-shelled  turtle (*Trionyx sinensis*) | Shenzhen,  China | Cultures | January 1997 | Not available | Typical clinical signs (neck swelling and haemorrhage of  the hypodermic area) – ‘red neck disease’ | Moderate.Mortality was less  than 40% (Challenge experiments) | Virus isolation, Electron microscopy  Challenge experiments | Chen *et al.*, 1999  Huang *et al.*, 2009 |
| \*Not specified | Alligator snapping turtles (*Macrochelys temminckii)* | Chengdu, Sichuan Province, China | Cultured  Pet  Introduced | March 2013 | Not available | crawled in weakness, slowed response to external stimulation, local redness and swelling in the neck and limbs | Not available | PCR, MCP gene sequencing and phylogenetic analysis | Yu *et al.,* 2015 |
| Grouper iridovirus (GIV) | Yellow grouper ( *Epinephelus*  *awoara)* | Hsiau Liouchiou Island, near Dunggang,  Taiwan. | Cultured | Not available | Not available | Not available | Not available | Virus isolation, Electron microscopy  Challenge experiments  PCR, MCP gene sequencing and phylogenetic analysis | Murali *et al.,* 2002; Lai *et al.,* 2000 |
| Ranavirus RCV-JP  (Similar to Rana catesbeiana virus) | North  American Bullfrogs  (*Lithobates catesbeianus,* formerly *Rana catesbeiana* | Western Japan | Wild/invasive (Introduced to Japan in 1918) | September – October 2008 | Imported infected live freshwater fish potentially from Taiwan | lethargy; palpebral hyperemia;  abdominal edema, petechiae,  and erythema on the ventral surface;  skin ulcers; limb and tail necrosis;  and emaciation. | Mass die-off | Histologic examination, Electron microscopy, PCR  MCP gene sequencing and phylogenetic analysis | Une *et al.,* 2009 |
| Inland bearded dragon ranavirus (IBDRV) | Inland bearded dragons (*Pogona vitticeps)* | Saitama Prefecture, Japan | Cultured  Pets  Introduced | December 2014 – February 2015 | Not available | Skin lesions with recurring multifocal yellowish-brown crusts | Approximately 50 individuals 9out of approxi. 100) had developed multifocal superficial crusting lesions and 15 had died | Histopathological examination, PCR,  MCP gene sequencing and phylogenetic analysis | Tamukai *et al.,* 2016 |
| \*Not specified  (Related to Rana catesbeiana virus JP MCP) | Huanren frog (*Rana huanrenensis*)  Tadpoles | Mountain stream in Injegun,  Kangwon-do, South Korea (37° 59′ 1.19′′N 128°29′24.70′′E | Wild | June 2015 | Not available | Skin of some tadpoles were slightly swollen | Mass mortality of tadpoles | PCR,  MCP sequence sequencing and phylogenetic analysis | Kwon *et al.,* 2017 |
| \*Not Specified  (MCP sequence highly resembled Frog virus 3 (FV3))  (same haplotype of a previously identified viral sequence collected from Huanren brown frog (*Rana huanrenensis*) from South Korea | Dybowski’s brown frog (*Rana dybowskii*) - Adults | Moksang-dong, Gyeyang-gu, Incheon, South Korea (37° 33′ 34.05″ N, 126° 42′ 12.79″ E) | Wild | March, 2017 | Not available | No distinctive external symptoms or erratic behaviors | Not available | PCR,  MCP gene sequencing and phylogenetic analysis | Park *et al.,* 2021 |
| Singapore grouper iridovirus (SGIV) | Grouper (*Epinephelus tauvina)* | Singapore | Cultured  Fry imported from other  SE Asian countries | 1998 | Fry were imported from other SE Asian countries and cultured in fish farms in Singapore. | Hemorrhage and enlargement of spleen | more than 90% mortality | Histopathological examination, PCR  MCP gene sequencing and phylogenetic analysis | Qin *et al.,* 2003 |
| Koi ranavirus (KIRV) | Koi (*Cyprinus rubrofuscus)* | South India | Cultured  Introduced | Published in 2015 | - | Skin darkening, loss of scales, vertical hanging, uncoordinated swimming, turning upside down, lateral rotation, intermittent surfacing, settling at the bottom laterally | mortality often  reached 100% | Virus isolation, Transmission electron microscopy, PCR  MCP gene  sequencing and phylogenetic analysis | George *et al*., 2015 |
| Similar damselfish virus’ (SRDV) (proposed)  (MCP gene showed  an identity of 99.82% to that of largemouth bass virus) | ‘‘Similar Damselfish’’ (*Pomacentrus*  *similis* Allen, 1991) | South India | Cultured  Marine ornamental fish | Not available | Not available | Haemorrhagic lesions, surface ulcerations and damaged caudal fin | 100% mortality  (challenge experiments) | Virus isolation, Electron microscopy  Challenge experiments  PCR, MCP gene sequencing and phylogenetic analysis | Sivasankar *et al*., 2017 |
| Tiger frog virus (TFV-1998) | Tiger frog  (*Hoplobatrachus*  *tigerinus*) | Bangkok, Thailand | Cultured | 1998 | Not available | Not available | Not available | Electron microscopy,PCR  MCP gene sequencing and phylogenetic analysis | Sriwanayos *et al.,* 2020 |
| *Oxyeleotris marmorata*  ranavirus (OMRV) | Marbled sleeper goby  (*Oxyeleotris marmorata*) | Nakhon  Pathom, Thailand | Cultured | 2000 | Not available | Skin lesions | Not available | Electron microscopy,PCR  MCP gene sequencing and phylogenetic analysis | Sriwanayos *et al., 2020* |
| *Poecilia reticulata ranavirus*  (PPRV) | Guppy (*Poecilia*  *reticulata*) | Samut  Sakhon, Thailand | Cultured  Ornamental  Introduced | 2001 | Not available | Not available | Not avaialble | Electron microscopy,PCR  MCP gene sequencing and phylogenetic analysis | Sriwanayos *et al., 2020* |
| Goldfish ranavirus  (GFRV) | Goldfish  (*Carassius*  *auratus*) | Bangkok, Thailand | Cultured  Ornamental  Introduced | 2002 | Not available | Not available | Not available | Electron microscopy,PCR  MCP gene sequencing and phylogenetic analysis | Sriwanayos *et al., 2020* |
| Asian grass frog  ranavirus (AGFRV) | Asian grass frog  (*Fejervarya*  *limnocharis*) | Sa Kaeo, Thailand | Cultured  Recently imported from Cambodia | 2004 | Probably infection originated in Cambodia | Cutaneous ulcerations | Not avialable | Electron microscopy,PCR  MCP gene sequencing and phylogenetic analysis | Sriwanayos *et al.,* 2020 |
| East Asian bullfrog  Ranavirus  (EABRV) | East Asian  bullfrog  (*Hoplobatrachusrugulosus)* | Phattalung/ Ratchaburi/Rayong, Thailand | Cultured | 2011, 2016, 2017 | Not available | ulcerative lesions on the dorsal part of the body and legs, cutaneous ulcerations and edema | Elevated  mortality | Electron microscopy,PCR  MCP gene sequencing and phylogenetic analysis | Sriwanayos *et al.,* 2020 |
| \*Not specified  Similar to largemouth bass virus  (LMBV) and identical to largemouth bass ulcerative syndrome virus (LBUSV) | Barcoo  Grunter (*Scortum barcoo)* | Ayutthaya and Phetchaburi  Provinces, Thailand | Cultured  Introduced from Australia | October to December, 2013 | Not available | Extensive haemorrhage and ulceration on skin and muscle | Up to 100% mortality | challenge assays,  PCR  MCP gene sequencing and phylogenetic analysis | Kayansamruaj et al., 2017 |
| Grouper iridovirus (GIV) | Tiger grouper hybrid (*Epinephelus sp*.) and  Coral trout (*Plectropomus leopardus*) | States of Selangor  and Kedah,  Peninsular Malaysia | Cultured  About 85 % fish fry are sourced mainly from Taiwan and  Thailand | 2012 to 2014 | Not available | Lethargy and darkening of  the tail and fins with focal distension of the skin and pale gills, necrosis of fins, sloughing of epidermis, dermal ulceration, enlarged spleen | Not available | Histopathology, PCR  MCP gene sequencing and phylogenetic analysis | Hazeri *et al.,* 2016  And  Hazeri *et al.,*2017 |