Příloha č. 1
Publikace, které jsou součástí disertační práce:

I. Fischer, D., Velenský P., **Chmelař, J.** & Rehák, I. (2016). European green lizard (*Lacerta viridis*) at the territory of Prague zoo. *Gazella* 43: 37-59.

Předkladatel disertační práce zpracoval shrnutí publikace v anglickém jazyce a podílel se na terénním sběru dat, která odevzdal školiteli prostřednictvím stručné zprávy.

II. **Chmelař, J.**, Civiš, P., Fischer, D., Frynta, D., Jeřábková. L. & Rehák, I. (2020). Distribution of the European green lizard, *Lacerta viridis* (Squamata: Lacertidae), in the Czech Republic: Real data and a predictive model. *Acta Societatis Zoologicae Bohemicae*, 84, 1-12.
Předkladatel disertační práce zpracoval je prvním autorem publikace, provedl statistickou analýzu dat a sepsal text manuskriptu včetně zpracování grafických výstupů.

III. **Chmelař, J.**, Civiš, P., Fischer, D., Frynta, D., Jeřábková., L, Rudolfová, V. & Rehák. I. (2023). Protecting isolated reptile populations outside their main area of distribution: a predictive model of the Dice snake, *Natrix tessellata*, distribution in the Czech Republic. *Biodiversity Data Journal*, 11**.**

Předkladatel disertační práce zpracoval je prvním autorem publikace, provedl statistickou analýzu dat a sepsal text manuskriptu včetně zpracování grafických výstupů, navíc byl i korespondenčním autorem a zapracovával připomínky v rámci recenzního řízení.

IV. **Chmelař, J.**, Frynta, D., Rudolfová, V., Fischer, D. & Rehák. I. (2024). Analysis of microhabitat preferences in the European green lizard (*Lacera viridis*) - a tool for conservation management of isolated populations. *Herpetozoa*. Date submitted: 12 February 2024, submission ID: 120806

Předkladatel disertační práce je prvním autorem publikace, provedl terénní sběr dat, jejich statistickou analýzu a sepsal text manuskriptu, navíc je i korespondenčním autorem a zajišťuje komunikaci a úpravy manuskriptu v rámci recenzního řízení.

V. **Chmelař, J.**, Civiš, P., Fischer, D., Frynta, D., Jeřábková., L, Rudolfová, V. & Rehák. I. (2024). Can predictive modelling of species distribution explain population decline? Study on the sand lizard, *Lacerta agilis*, in the Czech Republic. *Salamandra*. Date submitted: 23 February 2024, submission ID: 1668

Předkladatel disertační práce je prvním autorem publikace, provedl statistickou analýzu dat a sepsal text manuskriptu včetně grafických výstupů, navíc je i korespondenčním autorem a zajišťuje komunikaci a úpravy manuskriptu v rámci recenzního řízení.

VI. **Chmelař, J.**, Civiš, P., Fischer, D., Frynta, D., Jeřábková., L, Rudolfová, V. & Rehák. I. (2024). One snake to eat them all: A predictive model of *Coronella austriaca* distribution in the Czech Republic and its comparison to syntopic reptile species. *Amphibia-Reptilia*. Date submitted: 24 February 2024

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Ještěrka zelená ulovivší saranče (plocha č.5)
European green lizard hunting grasshopper (area No.5)
Foto/Photo Petr Velenský

Ještěrka zelená *(Lacerta viridis)* v areálu Zoo Praha

European green lizard *(Lacerta viridis)* at the territory of Prague zoo

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ÚVOD

V areálu Zoologické zahrady hl. m. Prahy se díky zvláštnímu režimu územního využití v pozoruhodné míře zachovala biologicky cenná společenstva svázaná s říčním fenoménem (srv. Rehák 2015). Projevuje se to i unikátními výskyty teplomilných živočichů, kteří se v okolní krajině mimo kaňon Vltavy nevyskytují. Jejich nápadným a z hlediska ochranářského významným reprezentantem je ještěrka zelená *(Lacerta viridis)*, která je u nás dle vyhlášky 395/1992 kriticky ohroženým druhem. V Čechách má ostrůvkovitý výskyt. České lokální populace jsou na severu druhového rozšíření – jako výsledek postglaciální holocenní expanze ještěrek zelených z jižnějších glaciálních refugií (Böhme et al. 2006a) - a jsou izolovány od souvislého druhového areálu i navzájem. Jejich izolovanost se odráží i v signifikantní genetické odlišnosti a nižší genetické variabilitě ve srovnání s populacemi ze souvislého areálu druhu (Böhme et Moravec 2005, srv. též Böhme et Berendonk 2005, Böhme et al. 2006b).

Populace ještěrky zelené v Zoo Praha má – jakožto izolovaná reliktní autochtonní populace - mimořádnou biologickou, vědeckou a ochranářskou hodnotu. Takové populace, vzhledem ke genetické výjimečnosti, související s izolovaností, fragmentací, malou populační početností, genetickým driftem, sníženou variabilitou a možností výskytu unikátních genetických variant, vyžadují i speciální metody ochranářského managementu (srv. Böhme et al. 2007, Joger et al. 2010). V posledních dekádách se situace ještěrek zelených v areálu pražské zoo opakovaně stala kritickou a je patrné, že bez aktivního ochranného managementu by mohla zcela zaniknout (Brantlová et al., 1991, Pecina 1992, 1993, 1998, Fischer et Rehák 2010, Fischer 2015, Rehák 2015).

V letech 2011 – 2015 byly provedeny v areálu Zoo Praha rozsáhlé managementové zásahy cílené na obnovu stepních biotopů v oblasti tzv. Zakázanky a následně další drobnější úpravy v letech 2016 a 2017, které výrazně změnily charakter dotčených částí. V tomto sdělení podáváme souhrn našich poznatků o stávajícím stavu populace ještěrky zelené v areálu Zoo Praha, včetně zhodnocení vlivu výše uvedených managementových zásahů na ještěrky zelené, a návrhy dalších opatření na její stabilizaci a posílení.

METODIKA

Výskyt ještěrek zelených v Zoo Praha je průběžně sledován autory práce a jsou k dispozici i pozorování dalších pracovníků Zoo Praha. Cílený intenzívní terénní výzkum byl proveden především v roce 2015 po rozsáhlých rekultivačních změnách jižního a jihozápadního svahu vltavského údolí, který má pro existenci místní populace ještěrky zelené zásadní význam, aby bylo možno zachytit dopad těchto změn na ještěrčí populaci (Fischer 2015). Následně byl autory prováděn průběžný monitoring.

Pro detailní výzkum v roce 2015 bylo vymezeno celkem sedm základních studijních ploch, v rámci kterých byl realizován monitoring. Hlavními kritérii pro výběr studijních ploch byly potvrzený výskyt zájmového druhu v minulosti, potenciální atraktivita plochy pro zájmový druh a vhodnost plochy pro následný monitoring z hlediska její přístupnosti a eliminace potenciálního nebezpečí pro návštěvníky (v podobě uvolněných kamenů v prudkém svahu). V důsledku toho byly z monitoringu vyřazeny i některé plochy, které jsou s největší pravděpodobností ještěrkami využívány. Např. část odlesněného nepřístupného svahu pod „Zakázankou" a část prudkého skalnatého svahu nad „Zakázankou".

Termíny jednotlivých návštěv byly voleny tak, aby postihly pokud možno nejvýznamnější etapy ročního cyklu aktivity ještěrek (např. období páření či období líhnutí mláďat) a zaručily co možná nejvyšší aktivitu ještěrek (volba vhodného počasí a času monitoringu). V rámci zvolených termínů bylo provedeno sčítání jedinců v jednotlivých studijních plochách. Těmi byl veden pozorovatelský transekt tak, aby byly prohledány pokud možno veškeré dostupné plochy potenciálně vhodné pro výskyt zájmového druhu. Zároveň byl při volbě vedení transektu a postupu v rámci jeho trasy kladen důraz na eliminaci možnosti vícenásobného započítání stejných jedinců (v případě jakýchkoliv pochybností nebylo dané pozorování bráno v potaz).

V rámci každé návštěvy byly (mimo jiné) zaznamenávány následující údaje: počasí, čas monitoringu, stav lokality (např. stav porostů, různé managementové zásahy), pozitivní i negativní jevy ve vztahu k ještěrkám a pozornost byla věnována i přítomnosti možných ještěrčích predátorů (např. kočky – včetně jejich fotodokumentace). Nálezy jednotlivých jedinců byly v terénu lokalizovány pomocí významných bodů (jednotlivé expoziční prvky, významné terénní prvky, stavby apod.) a následně zakresleny do leteckého snímku lokality.

Tab. 1: Přehled uskutečněných návštěv lokality v průběhu roku 2015 s uvedením bližších okolností monitoringu.

Tab. 1: Overview of site visits in 2015 - with monitoring details.

Datum	Zkoumané plochy	Čas	Počasí	Poznámky
27. 4.	1, 2, 4, 5	10.00 – 13.50	jasno, bezvětří, 17 – 19°C	primární návštěva, rekognoskace terénu, vymezení zájmových ploch; počátek vegetační sezóny, travinobylinná společenstva v rámci jedno-tlivých ploch nezapojená, mezer-natá a relativně nízkého vzrůstu
12. 5.	1 - 7	7.45 – 13.30	jasno, bezvětří, později středně silný vítr, 11 – 21°C	travinobylinná společenstva v rámci jednotlivých ploch stále ne zcela zapojená, částečně mezernatá; část plochy 2 prořezaná, probíhá vyklízení větví
18. 5.	1 (část), 2, 3, 4, 5, 6, 7	12.45 – 17.00	jasno, slabý vítr, 21°C	dtto, část plochy 4 čerstvě pokosená
5. 6.	1 (část), 4, 5, 6, 7	10.00 – 12.30	jasno, slabý vítr, 22 – 26°C	část plochy 1 zarostlá kompaktním porostem vysokých travin; biotopy na skalnatějších svazích značně proschlé v důsledku dlouhodobého srážkového deficitu
14. 7.	1 - 7	9.15 – 13.45	nejprve zataženo s deštěm, později přechodně polojasno, postupně oblačno, slabý vítr, 17°C	biotopy na svazích extrémně vyschlé (většina vegetace prakticky zcela „spálená") v důsledku dlouho-dobého srážkového deficitu a vysokých teplot; plocha 5 pečlivě vysekána
26. 8.	1 - 7	11.00 – 13.20	jasno, slabý vítr, 23 – 26°C	většina stanovišť (s výjimkou těch kolem vodních ploch) extrémně vyschlá v důsledku dlouhodobého srážkového deficitu a vysokých teplot (většina vegetace prakticky zcela „spálená")
5. 10.	1 - 7	11.45 – 14.30	jasno, slabý vítr, 18 – 24°C	většina stanovišť extrémně vyschlá (viz výše)

Nalezení jedinci byli rozděleni do následujících kategorií: adultní samci, adultní samice, adulti bez určení pohlaví, subadultní samci (po druhém přezimování), subadultní samice (po druhém přezimování), subadulti bez určení pohlaví (po druhém přezimování), 0++ jedinci (mláďata po prvním přezimování) a 0+ jedinci (tohoroční mláďata). Nalezení jedinci nebyli odchytáváni ani individuálně značeni. Příležitostně byla pořizována pouze fotodokumentace, která v některých případech umožňuje následnou identifikaci. V rámci zpracování získaných dat byl vyhodnocen i výskyt různých zaznamenaných traumatických a posttraumatických změn (mohou např. vypovídat o predačním tlaku).

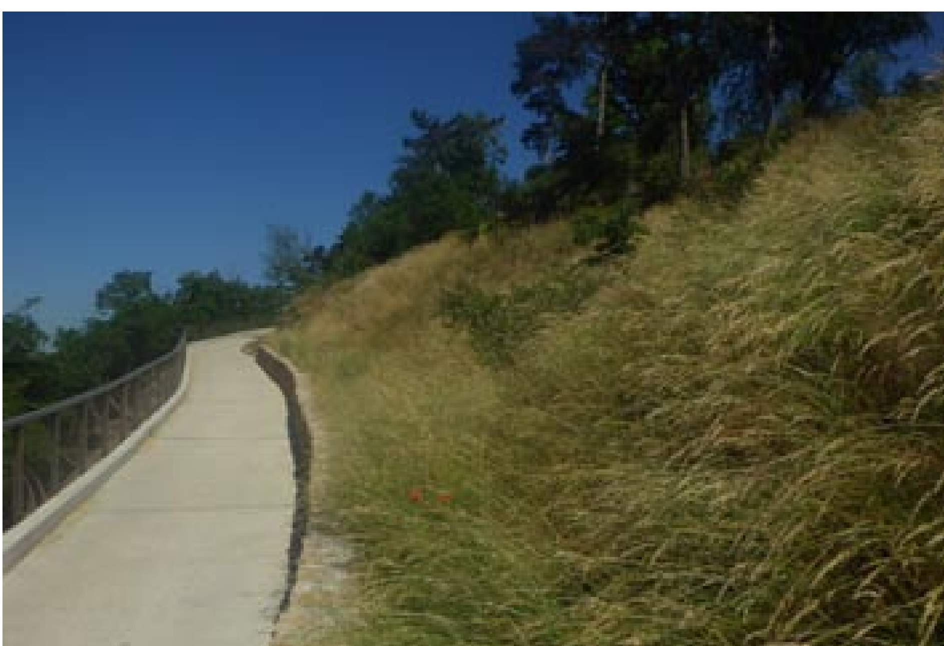
Na základě získaných výsledků byla graficky vyhodnocena prostorová distribuce výskytu ještěrky zelené v rámci zkoumaných studijních ploch. Dále byla zhodnocena i frekvence nálezů ještěrek v rámci zkoumaného souboru studijních ploch. Stanoveny byly i některé populační charakteristiky, jako je věková struktura či poměr pohlaví a byla hrubě odhadnuta i početnost místní populace a její hustota. Vzhledem k tomu, že jedinci nebyli individuálně rozpoznáváni, vycházel odhad početnosti populace v roce 2015 z nejvyšších počtů současně (během jednoho transektu) zaznamenaných a prokazatelně různých jedinců v jednotlivých sledovaných kategoriích a podílu zkoumaných ploch na celkové ploše stanovišť potenciálně vhodných pro výskyt ještěrek. I vzhledem ke skutečnosti, že se jednalo o vyhodnocení výsledků z jediné sezóny, navíc poznamenané extrémními suchy a dlouhodobě nadprůměrnými teplotami (což pravděpodobně vedlo k rapidnímu snížení aktivity ještěrek a snížení úspěšnosti líhnutí vajec), je třeba chápat provedený odhad početnosti populace pouze jako orientační údaj.

LOKALITA

V areálu Zoo Praha je populace ještěrky zelené vázána na jižně až jihozápadně orientované, místy skalnaté svahy centrální až JZ části zahrady, na nichž se zachovala specifická společenstva skalní stepi. Významná část těchto stanovišť je aktuálně součástí maloplošného zvláště chráněného území – PP Skály v zoologické zahradě – zřízeného Nařízením 17/2014 Sb. hl. m. Prahy s účinností od 1.12.2014. Příslušné svahy jsou velmi příkré a mají převýšení až 60 m. Vystupující srázy jsou tvořeny starohorními horninami (zejména šedé prachovce a břidlice s vložkami drob), je zde pozoruhodný odkryv ordovických vrstev s hrubě vrstevnatým slepencem a valouny a také nápadná přítomnost železné rudy. Nejen skalnaté srázy, ale i celý hřeben byly dle mapových podkladů z předminulého a minulého století až do padesátých let minulého století bez významnějších porostů stromů a keřů, avšak následně (zejména v šedesátých a sedmdesátých letech minulého století, jak ukazují letecké snímky) došlo k výraznému zarůstání svahů invazním akátem a dalšími dřevinami. Průběžně bylo provedeno několik managementových opatření k redukci nechtěných porostů, dostatečně radikální však byla až výše uvedená obnova z let 2011 – 2015. Pro bližší údaje k lokalitě viz Brantlová et al. 1991, Pecina 1992, 1993, 1998, Hrčka 2013, Fischer 2015, Rehák 2015, 2017.

Plocha 1: Jižně až jihozápadně orientovaný svah s výraznými skalními masivy, aktuálně téměř celoplošně pokrytými ochrannou drátěnou sítí (původně s výplní ok z degradovatelného plastu, která má ustoupit prorůstající vegetaci, což se již do značné míry stalo). Lokalita je ohraničena z jihu a jihozápadu nedávno rekonstruovanou zpevněnou cestou („Zakázanka“), ze severu pak taktéž zpevněnou cestou, tvořící jednu z páteřních návštěvnických tras. Svah, původně z větší části zarostlý zapojeným porostem akátu, popř. borovic, byl v letech 2011 – 2013 odlesněn za účelem rekonstrukce stepních biotopů (Rehák 2015). Tímto počinem byl dán základ pro vznik dalších vhodných stanovišť pro ještěrku zelenou na území zoo. Část je v současnosti téměř bez stromové a keřové vegetace (masivně zarůstá především travinami s dominujícím ovsíkem), na části je ponechán mozaikovitý porost keřů a nižších dřevin. Místy byly ponechány kmeny dřevin a vytvořeny palisády z kmenů a nahromaděných větví. Na jihu a jihozápadě navazuje prudký, zčásti taktéž nedávno odlesněný svah, který zhruba z poloviny zabírají otevřené výběhy. Druhá polovina nevyužívaného svahu je z velké části pokryta drátěným pletivem. Obě stanoviště odděluje nově rekonstruovaná cesta. Jeden její okraj tvoří kamenná zídka s vybetonovanými spárami, druhý pak vysoký hladký betonový obrubník. Tyto prvky jsou pro řadu druhů živočichů (např. obojživelníci) jen obtížně překonatelnou či nepřekonatelnou migrační bariérou. Částečně tato bariéra ztěžuje migraci i plazům, včetně zájmového druhu. V ploše 1 jsou zbudovány malé venkovní expozice našich plazů. Na východě svah pokračuje čerstvě odlesněnými plochami, na kterých

Střední část plochy 1(5.10.2015). Z pohledu ještěrek jedna z nejatraktivnějších partií zoo
Middle part of area 1 (5.10.2015). From the view of lizards, one of the most attractive areas of the zoo
Foto/Photo David Fischer



Severozápadní část plochy 1 (5.6.2015)
Northwest part of area 1 (5.6.2015) Foto/Photo David Fischer

Střední část plochy 1(5.10.2015). Z pohledu ještěrek jedna z nejatraktivnějších partií zoo
Middle of area 1 (5.10.2015). From the view of lizards, one of the most attractive areas of the zoo
Foto/Photo David Fischer

byla v roce 2015 zbudována vinice (i tato plocha by za předpokladu, že bude citlivě obhospodařována a vhodně doplněna určitými prvky mohla do budoucna sloužit jako biotop ještěrky zelené). Cca 60 % plochy 1 je součástí PP Skály v zoologické zahradě.

Plocha 2: Část jihozápadně exponovaného svahu, navazujícího na severozápadní cíp plochy 1 (obě části lokality odděluje zpevněná cesta – „Zakázanka"). Zkoumána byla pouze horní polovina svahu (spodní část je velmi prudká, zčásti zasíťovaná drátěným pletivem, mnohdy ale s volnými kameny a sutí, která by při pohybu touto částí lokality mohla být uvolněna a mohla by tak zranit návštěvníky zahrady). Svah byl zčásti čerstvě odlesněn (v jarním období 2015 odtud byly vynášeny zbytky větví) a v průběhu jara 2015 tu probíhala chemická likvidace zmlazujících akátů. Část svahu (pod rozhlednou) je zarostlá poměrně zapojeným, nicméně řídkým porostem křovin s řídkým travinobylinným podrostem. Části svahu jsou téměř bez vegetačního krytu, severní a severovýchodní část plochy naopak zarůstá bujným porostem travin. Většina plochy je součástí PP Skály v zoologické zahradě

Plocha 3: Příkrý svah lemující cestu ohraničující ze severu část plochy 1. Jedná se o úzký, dobře osluněný (jižně exponovaný) lem cesty s mezernatou vegetací a skalními výchozy (navazující část svahu je ale stinná, s kompaktním porostem křovin a dřevin). Do svahu jsou zakomponovány voliéry. Ještěrky zde mohou volně migrovat do navazující plochy 1.

Plocha 4: Pata JZ exponovaného svahu se skalnatými výchozy a suťovými poli, většinou ale poměrně hustě zarostlá dřevinami a křovinami s přilehlou plošinou s tenisovým kurtem a malou budovou, s kosenými trávníky na severozápadě a nekompaktními porosty dřevin s travinobylinným podrostem v jihovýchodní části (zde i zbytky základů bývalých staveb). Jedná se o mimořádně významné stanoviště pro užovku podplamatou (probíhá zde její páření), jejíž volně žijící populace rovněž obývá areál pražské zoo. V průběhu roku 2015 byla v návaznosti na jihovýchodní část plochy zahájena výstavba nových budov.

Plocha 5: Plochá proluka po bývalém pavilonu šelem při patě jižně exponovaného skalnatého svahu (pokryt drátěnou sítí). Plocha je tvořena stropem kanalizačního přivaděče. Okraje zarůstají kompaktním porostem křovin. Před úpravami v roce 2016 většinu plochy pokrývala řídká travinobylinná vegetace

Charakter plochy 3 (5.10.2015)
Appearance of area 3 (5.10.2015)

Foto/Photo David Fischer

Charakter plochy 5 (18.5.2015)
Appearance of area 5 (18.5.2015) Foto/Photo David Fischer

(porost byl kosen). Ojediněle byly přítomny větší kameny. Část volného prostranství sloužila v roce 2015 jako dočasná deponie větví. V roce 2016 byla tato plocha rekultivována tak, aby si zachovala vysokou heterogenitu prostředí a aby vzrostla její využitelnost pro ještěrky zelené. Pata skály byla obohacena valem ze skládaných kamenů, na ploše vznikly dvě foliové tůně, zbylá plocha byla oseta luční vegetací. Od návštěvního prostoru odděluje přírodní stanoviště nízká skládaná zídka. Velmi dobře osluněná část lokality, oddělená na jihu asfaltovou cestou od plochy 6. Na západě i východě sousedí s venkovními expozicemi a představuje tak pravděpodobně velmi významný migrační koridor, umožňující populaci ještěrek komunikovat se stanovišti v jižní části zahrady, mimo jiné i s plochou 6.

Plocha 6: Jižně až jihozápadně exponovaný břeh vodního příkopu, při východním okraji s krátkou skládanou zídkou. Místy dobře osluněný, místy zastíněný. Různé typy vegetačního krytu, od mokřadních druhů v lemu vodní plochy, přes řídké travinobylinné porosty, po prakticky holé enklávy. Část břehu je porostlá břečťanem. Okraj svahu se vzrostlými dřevinami. Na severu a severovýchodě je plocha oddělena od navazujících biotopů (mimo jiné plocha 5) asfaltovou cestou.

Plocha 7: Úzký lem mezi skalním masivem a asfaltovou veřejnou cestou (jedna z páteřních a velmi frekventovaných tras). Mezernaté porosty dřevin a křovin, přes jižní expozici je plocha poměrně stinná. V lemu cesty výsadby okrasných keřů, část plochy překryta štěpkou. Místy řidší travinobylinné porosty. Na severu navazují skalní biotopy veskrze překryté drátěnou ochrannou sítí.

Populační disperze: Ještěrky byly zaznamenány ve všech vymezených studijních plochách. V roce 2015 byla jednoznačně nejvyšší frekvence výskytu zjištěna v ploše 1 (52,1 % všech záznamů), s 29,6 % záznamů pak následuje plocha 5. Jedinou studijní plochou, v rámci které byl zaznamenán výskyt ještěrek při každé provedené kontrole, byla plocha 1, což vyplývá, mimo jiné, i ze skutečnosti, že se jedná o největší studijní plochu s nejvyšší heterogenitou. Frekvence záznamů ještěrek v rámci ostatních zkoumaných ploch se pak pohybovala v rozmezí od 1,4 % – 5,6 % a ještěrky zde byly pozorovány pouze v rámci některých návštěv (výskyt zde nebyl kontinuální). Tyto plochy jsou využívány pravděpodobně spíše příležitostně – v roce 2015 mohl být např. přesun ještěrek do blízkosti vodního kanálu (plocha 6) důsledkem extrémního sucha a dlouhodobě trvajících vysokých teplot.

Kromě plochy 2, resp. 4, a plochy 1, kde byly na skalách pod lanovkou ještěrky pozorovány jako na jedné z posledních ploch před zahájením rozsáhlých managementových prací v roce 2011 (Pecina 1992) a pak až od roku 2014 (Rehák 2015), jsou záznamy výskytu ještěrek v ostatních studijních plochách pravděpodobně prvními novodobými pozorováními jejich výskytu v těchto částech Zoo. Nízké počty pozorovaných jedinců v některých studijních plochách jsou ale přinejmenším překvapivé. Např. plocha 2, která je podle pozorování v minulosti považována za klíčovou pro existenci místní populace ještěrek, kam byly již v minulosti cíleny managementové zásahy na podporu tohoto druhu (Pecina 1992, 1993, 1998) a ještě poměrně nedávno (kolem roku 2010) zde byly během jediného dne pozorovány desítky jedinců (např. Víta in verb. uvádí z roku 2010 jednorázové pozorování cca 25-ti adultních samců, z let 2009 – 2011 pak i pozorování více než 30-ti různých jedinců). V roce 2015 zde byla ale ještěrka zastižena pouze 2 x (adultní samice, 0++ jedinec).

Subadultní samice – plocha 3
Subadult female - area 3

Foto/Photo David Fischer

Adultní samec v době páření – plocha 1
Adult male at mating season - area 1 Foto/Photo David Fischer

O příčině tohoto stavu lze pouze spekulovat. V roce 2015 zde byl např. prováděn management (vyřezávání keřů, aplikace herbicidů). Na jedné straně se mohlo jednat o možnou příčinu přesunu ještěrek do jiné části lokality (zvýšené rušení, aplikace chemických prostředků?), na straně druhé zde zůstala značná část plochy bez uvedených zásahů a ani zde ještěrky nalezeny nebyly. Dokonce zde přechodně vznikala velmi vhodná stanoviště např. ve formě hromad větví, které na jiných místech ještěrky hojně využívaly (např. plocha 5). Co se týká rušení ještěrek v důsledku zvýšeného pohybu osob, není tato skutečnost jako příčina jejich vymizení z dané části lokality příliš pravděpodobná. Na jiných místech zahrady totiž ještěrky využívají plochy v bezprostřední blízkosti frekventovaných cest (např. plochy 1, 3, 5, 6) nebo plochy, které jsou využívány k dočasné deponaci větví (plocha 5). Stejně tak naše zkušenosti z jiných lokalit ukazují, že i velmi razantní managementové zásahy na lokalitách s výskytem tohoto druhu nevedou k podobnému efektu – naopak ještěrky okamžitě využívají např. padlé kmeny nebo dočasné kupy větví jako přechodná stanoviště.

Podobně překvapivý je pouze jediný nález ještěrky v ploše 4, která z jihu navazuje na plochu 2 (je od ní oddělena cestou), a která nabízí řadu potenciálně atraktivních stanovišť (mimo jiné se jedná o významnou plochu z pohledu místní populace užovky podplamaté, která zde byla i v průběhu roku 2015 běžně pozorována).

Dalším možným vysvětlením zjištěného stavu je relativně čerstvý managementový zásah (ukončení v roce 2013) kolem „Zakázanky" (plocha 1 a navazující území), který nabídl ještěrkám místy velmi atraktivní nové biotopy a nové migrační možnosti. To mohlo mít sice za následek přesun části populace do těchto obnovených stanovišť, nicméně je velmi nepravděpodobné, že by došlo k takto rychlé emigraci převážné většiny jedinců, navíc za situace, kdy se plocha 2 ani plocha 4 zdaleka nenacházejí ve stavu, který by byl pro ještěrku zelenou neatraktivní.

Další možností je přesun části jedinců do partií, které nebyly předmětem monitoringu – např. části svahu přiléhající k ploše 2 z jihu a jihozápadu, popř. skutečnost, že se v rámci monitorovaných ploch jedná o biotop, který je nejčlenitější a nejméně přehledný. Část jedinců tak mohla v rámci monito-

Adultní samice – plocha 1
Adult female - area 1 Foto/Photo David Fischer

ringu uniknout pozornosti. Vysvětlení výše uvedené skutečnosti lze s největší pravděpodobností hledat v kombinaci všech výše uvedených faktorů. V budoucnu by měla být tomuto zajímavému jevu každopádně věnována zvýšená pozornost.

Podle pozorování v roce 2016 lze konstatovat pozitivní vývoj plochy č. 1 v důsledku sukcese spojené s mírným zarůstáním lokality. Rovněž úpravy provedené na ploše č. 5 jsou pro ještěrky zřejmě přínosné. V obou případech bude potřebné v následujícím období situaci na obou těchto plochách monitorovat a průběžně vyhodnocovat. Na ostatních plochách nebyly zaznamenány žádné významnější změny.

Prostorové využití jednotlivých sledovaných ploch ještěrkami je velmi nerovnoměrné, ještěrky využívají pouze určité jejich části. Podobný charakter disperze zaznamenali Fischer et Rehák (2010) i u populace ještěrek zelených v Tichém údolí na protilehlém břehu Vltavy. Nejlépe je tato skutečnost patrná v rámci plochy 1. Významným kritériem výběru vhodných míst je pro ještěrky evidentně úkrytová kapacita daných částí lokality (Blažek 2013, Chmelař 2013, 2016). V ploše 1, kde byl v období 2011 – 2013 proveden razantní management, který značné části lokality zcela zbavil porostů dřevin , je největší frekvence nálezů soustředěna do několika jasně vymezených zón.

První je oblast kolem voliéry a nevyužívané cesty (schodiště) v severovýchodní části sledované plochy. V dané části se nalézá řada potenciálních úkrytů v porostech plazivých keřů, prostorách pod kořeny poražených stromů, popř. ve skládaných zídkách podpírajících zpevněnou cestu. Místy zde také byly v rámci managementu lokality ponechány kmeny poražených stromů či formace z nahromaděných větví.

Další významnou zónou jsou části lokality pod lanovou dráhou, kde měl i původně porost jiný charakter, a mohl zde tak být ponechán mozaikovitý porost nižších křovin. Kromě toho se zde vyskytují i keře ostružiníku, jsou tu umístěny různé technické prvky (schodiště pod lanovkou) a skalní výchozy

Pár při námluvách – plocha 5
Couple at courtship - area 5
Foto/Photo David Fischer

jsou překryty drátěným pletivem, využívaným ještěrkami jako úkryt. Místy jsou ve svahu ponechány i hromady větví a terén je zde obecně velmi členitý. Podobný charakter mají i části navazujících ploch při horní hraně svahu (ponechané kmeny, mozaikovitý výskyt nižších křovin).

Poslední významnější zónou s vyšší frekvencí výskytu ještěrek je pak úzký pás mezi kamennou zídkou lemující „Zakázanku" a prudkou hranou svahu s pařezy poražených stromů, rozpadajícím se skalním výchozem, popř. skalním výchozem pokrytým drátěným pletivem.

Veškeré uvedené plochy mají dva pro ještěrky významné atributy: jsou dobře osluněné a mají vysokou úkrytovou kapacitu – jinými slovy nabízejí vhodná místa ke slunění v blízkosti potenciálních úkrytů. Jako úkryty v daném případě ještěrky využívají prostory pod pařezy, hustou plazivou či polehlou vegetaci, až k zemi zavětvené keře, prostory pod drátěnou ochranou skalních masivů, prostory pod polehlými kmeny či hromady větví, pukliny a prostory v rozpadající se skále či erodujícím terénu a nory vyhloubené drobnými savci. Jako místa ke slunění pak širokou škálu stanovišť v blízkosti potenciálních úkrytů (osluněné plochy volného terénu, umělé zídky, betonové schody u voliéry, okraje skalních výchozů, ponechané kmeny stromů, pařezy či kořenové náběhy pokácených dřevin nebo ponechané hromady větví).

Značná část plochy 1 ale v současné době žádoucí kombinaci vhodných míst ke slunění a dostatků atraktivních úkrytů nenabízí – podstatná část je zcela holá (pouze s travinobylinnou vegetací), na dalších plochách nejsou keře zavětveny až k terénu; pod pařezy poražených dřevin nejsou dostatečné prostory (pokud jsou, jsou ještěrkami využívány – viz nejseverněji prokázaný výskyt v rámci plochy 1). Závislost výskytu ještěrek na přítomnosti vhodných úkrytů je dobře patrná i např. v případě plochy 5. Zde byly ještěrky (v hojném počtu) pozorovány v době, kdy zde docházelo k nárůstu travinobylinné vegetace (kryt při pohybu jinak otevřenou plochou proluky), a kdy zde byly deponovány na hroma-

0+ jedinec využívající umělou skládanou zídku – plocha 6
0+ individual using artificial wall of stacked stones - area 6 Foto/Photo David Fischer

dách větve. Od chvíle, kdy bylo dno plošiny pečlivě vysekáno (kolem 14.7.2015, načež zde v důsledku extrémních teplot a sucha již vegetace dostatečně nenarostla) a byly odstraněny hromady větví, nebyl zde již výskyt ještěrek vůbec zaznamenán.

Věková skladba populace: Ať již odvozená od počtu nalezených prokazatelně různých jedinců, tak od frekvence nálezů jednotlivých věkových kategorií, koresponduje s údaji zjištěnými u populace v Tichém údolí Fischerem et Rehákem (2010). Na lokalitě v Tichém údolí byly těmito autory zjištěny v různých letech poměry jednotlivých věkových kategorií (adult: subadult : juvenil bez dalšího rozlišení na 0+ a 0++) 1 : 0,21 : 0,68; 1 : 0,33 : 0,70; 1 : 0,34 : 0 a 1 : 0 : 0,46. Zjištěné poměry byly přitom jednoznačně závislé na úspěšnosti reprodukce ještěrek v jednotlivých letech. Pokud by byly v případě výsledků, získaných v roce 2015 při sledování populace v Zoo Praha, sloučeny kategorie 0+ a 0++, vycházejí poměry jednotlivých kategorií v případě, že jsou odvozeny od počtu prokazatelně různých zaznamenaných jedinců 1 : 0,12 : 0,65, v případě, že jsou odvozeny od frekvence pozorování jednotlivých věkových kategorií pak 1 : 0,16 : 0,45. Tyto hodnoty dobře korespondují s hodnotami zjištěnými v Tichém údolí.

Poměr pohlaví: Vyrovnaný - pohybuje zhruba kolem hodnoty 1 : 1. V případě, že je odvozen od počtu prokazatelně různých zaznamenaných jedinců, dosahuje hodnoty 0,88 : 1 ve prospěch samic, v případě, že je spočten na základě frekvence pozorování jednotlivých pohlaví, dosahuje hodnoty 1,33 :1 ve prospěch samců. Fischer et Rehák (2010) zaznamenali u nedaleké populace v Tichém údolí poměr pohlaví v různých letech v rozmezí 1,08 – 1,38 : 1 ve prospěch samců, což v podstatě koresponduje se zjištěnými hodnotami v rámci populace v Zoo Praha.

Početnost populace: Lze v daném případě pouze hrubě odhadovat. V rámci provedených průzkumů bylo na lokalitě zaznamenáno 30 prokazatelně různých jedinců. Vzhledem ke skutečnosti, že předmětem průzkumu byly odhadem necelé 2/3 ploch potenciálně vhodných pro ještěrky, které jsou

0++ jedinec, využívající skalní výchoz překrytý drátěnou sítí – plocha 1
0 ++ individual using a rock outcrop covered by wire mesh - area 1 Foto/Photo David Fischer

v areálu Zoo k dispozici, a s přihlédnutím ke způsobu získání aktuálních dat (pouze sedm celodenních pozorování, průzkum prováděný během jediné sezóny s poměrně netypickým průběhem počasí, neznačkování jedinců), může být skutečná početnost místní populace výrazně vyšší – kvalifikovaným odhadem přinejmenším cca dvojnásobná. Vyšší skutečné početnosti napovídají i pozorování Víty (in verb.), kdy v rozmezí let 2009 – 2011 v rámci plochy 2 a přilehlých stanovišť pozoroval jednorázově např. cca 25 adultních samců. Z téhož období uvádí ze stejných ploch jednorázová pozorování i více než 30-ti různých jedinců (včetně juvenilů).

Hustota populace: Při ploše zkoumané části lokality cca 2 ha se pohybuje kolem 15 jedinců /ha (ve skutečnosti může být – stejně jako početnost – vyšší), což koresponduje s populační hustotou zjištěnou na nedaleké lokalitě v Tichém údolí, která se pohybovala v rozmezí 11 – 22 jedinců / ha (Fischer et Rehák 2010).

Traumatické a post-traumatické změny: V rámci provedených pozorování byl zaznamenán regenerát ocasu u dvou adultních samců a jednoho 0+ jedince. To představuje 6,7 % jedinců za situace, kdy je podíl spočten z počtu zaznamenaných prokazatelně různých ještěrek, popř. pouze 4,2 % ze všech pozorovaných jedinců během celého roku 2015. Pro srovnání – u nedaleké populace v Tichém údolí byl Fischerem et Rehákem (2010) zaznamenán výskyt regenerátu u celkem 26 % jedinců! Nízké procento výskytu regenerátů u ještěrek ze sledovaných ploch v rámci Zoo Praha může signalizovat nižší predační tlak. Na druhou stranu může znamenat také pouze skutečnost, že jsou zde ještěrky predátory loveny úspěšněji, než je tomu na lokalitě v Tichém údolí. Nižší procento výskytu regenerátů, zejména u samců, může taktéž signalizovat nižší hustotu populace (tím i méně příležitostí, kdy dochází např. k soubojům samců a vzájemnému zraňování) – vzhledem ke zjištěné kumulaci populace do několika výrazných okrsků i vzhledem ke skutečnosti, že populační hustota je s hodnotami zjištěnými v Tichém údolí srovnatelná, je však tato interpretace pouze málo pravděpodobná.

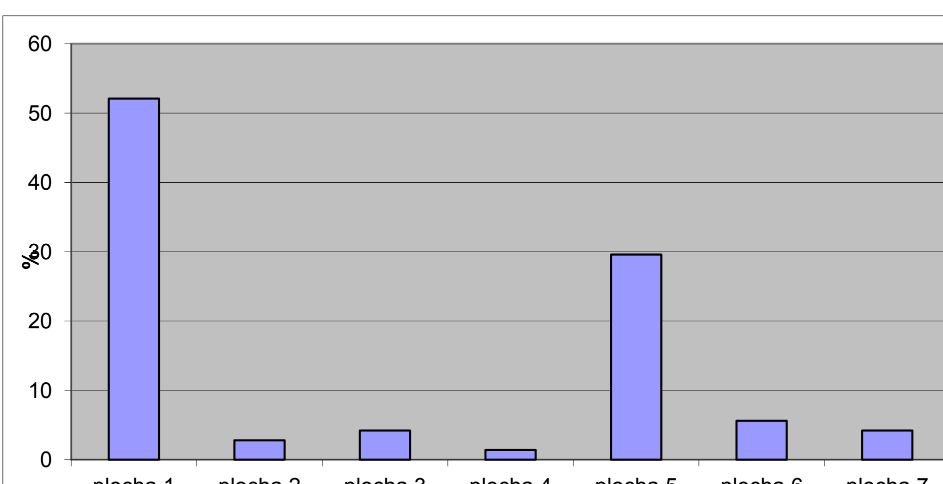
Tab. 2: Frekvence pozorování ještěrek zelených (včetně rozdělení na jednotlivé kategorie) ve sledovaných studijních plochách v průběhu monitoringu realizovaného v roce 2015.

Tab. 2: Frequency of observation of green lizards (including division into individual categories) in monitored study areas during the monitoring carried out in 2015.

Kate-gorie	Plocha 1	Plocha 2	Plocha 3	Plocha 4	Plocha 5	Plocha 6	Plocha 7	Celkem	
ad. samci	7	0	0	1	6	1	1	16 = 22,5 %	62%
ad. samice	8	1	0	0	3	1	1	14 = 19,7 %	
ad. neid.	9	0	0	0	4	1	0	14 = 19,7 %	
subad. samci	0	0	0	0	1	0	0	1 = 1,4 %	9,8 %
subad. samice	0	0	1	0	0	0	0	1 = 1,4 %	
neid. subad.	4	0	0	0	1	0	0	5 = 7 %	
0+	1	0	2	0	0	1	0	4 = 5,6 %	
0++	8	1	0	0	6	0	1	16 = 22,5 %	
celkem	37 = 52,1 %	2 = 2,8 %	3 = 4,2 %	1 = 1,4 %	21 = 29,6 %	4 = 5,6 %	3 = 4,2 %	71	

Graf 1: Frekvence pozorování ještěrek zelených ve sledovaných studijních plochách v průběhu monitoringu realizovaného v roce 2015.

Figure 1: Frequency of observation of green lizards in monitored study areas during the monitoring carried out in 2015.



Mapa 1: Lokalizace a frekvence nálezů ještěrek zelených v rámci monitoringu provedeného v průběhu roku 2015 – souhrnné výsledky ze všech provedených návštěv. Mapový podklad: www.mapy.cz

Map 1: Localization and frequency of Green Lizard findings during the 2015 monitoring - aggregate results from all visits made. Map background: www.mapy.cz

Tab. 3 Shrnutí výsledků monitoringu ještěrky zelené v roce 2015 ve vybraných studijních plochách. A ... adult; Sba ... subadult; 0+ ... juvenil tohoroční; 0++ ... juvenil po 1. přezimování
Tab. 3 Summary of green lizards monitoring results in 2015 in selected study areas. A ... adult; Sba ... subadult; 0+ ... hatchling; 0 ++ ... juvenile after the 1st hibernation

Datum (2015)	Zkoumané plochy	Plocha 1								Plocha 2								Plocha 3								Plocha 4							
		A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++
27.4.	1, 2, 4, 5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	nezkoumána								0	0	0	0	0	0	0	0
12.5.	1 - 7	5	6	0	0	2	0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.5.	1 (část), 2, 3, 4, 5, 6, 7	0	0	0	0	2	0	0	0																	1	0	0	0	0	0	0	0
5.6.	1 (část), 4, 5, 6, 7	0	1	0	0	2	1	0	3	nezkoumána								nezkoumána								0	0	0	0	0	0	0	0
14.7.	1 - 7	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
26.8.	1 - 7	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.10.	1 - 7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0

Datum (2015)	Zkoumané plochy	Plocha 5								Plocha 6								Plocha 7								Celkem								
		A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	A ♂	A ♀	Sba ♂	Sba ♀	A neid.	Sba neid.	0+	0++	celkem
27.4.	1, 2, 4, 5	1	0	1	0	2	0	0	1	nezkoumána																2	1	1	0	2	0	0	1	7
12.5.	1 - 7	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	8	0	0	2	0	0	6	23
18.5.	1 (část), 2, 3, 4, 5, 6, 7	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	2	0	0	2	8
5.6.	1 (část), 4, 5, 6, 7	1	1	0	0	2	1	0	3	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	2	3	0	0	4	2	0	7	18
14.7.	1 - 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	0	0	5
26.8.	1 - 7	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	2	0	0	6
5.10.	1 - 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4

I přes skutečnost, že díky provedenému managementu se z pohledu ještěrky zelené jednoznačně silně zvýšila atraktivita řady ploch v rámci zoologické zahrady a došlo díky tomu k expanzi tohoto druhu na mnohem větší území, byly zaznamenány některé jevy s negativními, popř. potenciálně negativními dopady na místní populaci tohoto druhu:

Nízká úkrytová kapacita: Zaznamenána v některých částech nově rekonstruovaných stanovišť. Management, realizovaný na stanovištích obývaných ještěrkami, lze obecně vnímat velmi pozitivně, avšak v některých případech byl prováděn až příliš razantně. Např. v rámci části plochy 2, pro místní populaci ještěrky zelené za mimořádně významné, bylo zaznamenáno místy plošné odstranění stromového a keřového krytu s následným vyklizením prakticky veškerého vyřezaného materiálu. Větší zcela holé plochy se stávají z pohledu ještěrek neatraktivními díky absenci potenciálních úkrytů, za které jim velmi často slouží např. nízko zavětvené keře. Vzhledem k jižní expozici daných svahů pak navíc dochází k situaci, že zde zcela chybí zastíněné plochy důležité pro termoregulační chování ještěrek a svahy pak navíc v letním období extrémně vysychají.

Odlišná situace (s na první pohled podobným výsledkem) nastala v případě plochy 1. Vzhledem k výchozímu stavu obnovovaných stepních lokalit, které byly ze značné části porostlé hustými porosty dřevin s převahou akátu – a pro ještěrky zcela neatraktivní – musel být primární managementový zásah realizován velmi razantně. Některé části lokality (zejména severozápad) tak musely být zcela zbaveny hustého porostu dřevin (bez možnosti ponechání mozaiky vhodných keřů) a keře ponechané v jiných partiích nejsou vzhledem k podmínkám, ve kterých původně vyrůstaly, zavětveny až k zemi. Nedostatek úkrytů po provedeném zásahu byl částečně velmi vhodně kompenzován vybudováním umělých prvků, jako jsou ponechané ležící kmeny stromů či formace z větví a slabších kmínků. I přes tyto snahy zde ovšem, zejména v severozápadní části plochy, zůstaly partie s velmi nízkým úkrytovým potenciálem, které nebyly v roce 2015 ještěrkami pravděpodobně vůbec využívány. K jejich kumulaci pak docházelo v těch částech lokality, kde byl primární zásah patrně šetrnější (okolí lanové dráhy), popř. tam, kde byly zachovány či vytvořeny podmínky s vyšší úkrytovou kapacitou.

Přes výše uvedené dílčí výhrady byl z pohledu ještěrek zelených provedený management zcela zásadním pozitivním krokem, o čemž svědčí rychlá kolonizace dané plochy větším množstvím jedinců (první jedinci zde byli pozorováni prakticky bezprostředně po dokončení prací již v roce 2014). Podobný efekt rychlého osídlení nově obnovených ploch byl zaznamenán i v rámci plochy 2 a přilehlých částí lokality při prvních rozsáhlejších pokusech o záchranu populace ještěrky zelené na území Zoo Praha (Pecina 1992, 1993).

Migrační bariéry: V rámci rekonstrukce cesty vedoucí napříč jižně exponovaným svahem, jehož součástí je i studijní plocha 1 („Zakázanka"), byly při jejím okraji zbudovány vysoké hladké betonové obrubníky. V kombinaci s hladkou betonovou patkou cesty, vysokou mnohdy i kolem 2 m, se tak tato pěší komunikace stala bariérou komplikující migraci drobných živočichů tímto svahem. Do jaké míry znemožňuje tento prvek migraci právě ještěrek zelených na základě získaných dat nelze jasně vyhodnotit, migrační překážkou i pro tento druh však zcela jistě může být.

Zvýšený predační tlak: V rámci realizovaných návštěv lokality byly v plochách s výskytem ještěrek či v jejich bezprostředním okolí často pozorovány kočky, kterých na území Zoo Praha žije pravděpodobně poměrně značné množství. Kočky přitom mohou pro ještěrky představovat velmi významné nebezpečí – mohou je lovit jak jako zdroj potravy, tak např. i v rámci her (tento aspekt může být i vysvětlením nálezu zabitého, ale jinak neporušeného adultního samce v ploše 2 v roce 2014). Lov ještěrek různých druhů v rámci her (tedy aniž by pak byly pozřeny) je u koček pravděpodobně poměrně běžným jevem a např. v okolí vesnických lidských sídel je často hlavním důvodem výrazného snížení početnosti, v extrémních případech i vymizení těchto živočichů (Fischer, vlastní pozorování).

Nad lokalitou pravidelně loví poštolky obecné *(Falco tinnunculus)*. Je jediným predátorem, u něhož byla predace na ještěrku zelenou doložena přímým pozorováním - nález ulovené ještěrky zelené v hnízdě poštolky na římse bývalého pivovaru (Velenský, vlastní pozorování). Na lokalitě se zdržuje množství strak obecných *(Pica pica),* které považujeme téměř s jistotou za vážné predátory ještěrky zelené, a to podle pozorování, kdy doslova pročesávají a prohrabávají vrchní vrstvy půdního profilu, což interpretujeme jako lov mláďat ještěrek. Kůs (in lit.) jednou pozoroval na skalním ochozu pod Oborou straky peroucí se o ještěrčí kadaver.

V areálu Zoo Praha se vyskytuje i řada dalších potenciálních predátorů. Žije tu např. užovka hladká *(Coronella austriaca)*, která je saurofágní, nicméně na území Zoo Praha byla za posledních pět let pozorována jen čtyřikrát (a jen dvakrát přímo na lokalitě ještěrky zelené). To by nasvědčovalo nízké lokální abundanci tohoto hada, která ovšem může být právě interkorelována s nízkou abundancí ještěrek. Nebezpečí mohou představovat pro ještěrku některé další druhy ptáků (byl zde pozorován i bažant obecný, *Phasianus colchicus*). V bezprostřední blízkosti ploch s výskytem ještěrek vznikla po rekultivaci hnízdní kolonie volavek popelavých *(Ardea cinerea)*, nebylo ovšem doloženo, že by jimi byly ještěrky loveny. Kůs (in lit.) popisuje, jak vídával nad strání pod oborou a nad bývalými „Gočáry" pávy korunkaté *(Pavo cristatus)*, kteří se volně pohybují v areálu Zoo Praha: „Chodili tam docela pravidelně a pročesávali otevřená místa, jednoho jsem osobně viděl jak ještěrku ulovil". Ze savců žijících na území zoo mohou pro ještěrku představovat hrozbu např. ježci, potkani, lasicovité šelmy, lišky a divoká prasata. 5.10.2015 byl zaznamenán v místech výskytu ještěrek (popř. v místech jejich možných zimovišť) větší počet vyhrabaných nor (průměr cca 8 – 10 cm). Původce se bohužel nepodařilo identifikovat, nicméně může se jednat o výsledek snahy savčích predátorů o predaci zimujících ještěrek, popř. užovek podplamatých. V těchto souvislostech byl pozoruhodný nález (ještě před ukončením období přezimování) ještěrky zelené, která byla evidentně vyhrabána a usmrcena nějakým predátorem.

Predaci ze strany výše uvedených živočichů (s výjimkou koček a potkanů) lze sice považovat za přirozenou, ale při nízké abundanci ještěrek může být její dopad na populaci velice závažný.

Používání herbicidů: Při následné údržbě vykácených ploch jsou využívány herbicidy (Roundup), což je při managementu tohoto typu běžné (např. lokální aplikace na řez, ale i na list). V případě části plochy 2 byly ale v roce 2015 tyto látky na výmladky stromů zřejmě aplikovány postřikem a ve značném množství. V rámci takovéhoto způsobu užití je pak samozřejmě likvidován i další vegetační kryt v okolí likvidovaných dřevin (což může v důsledku vést např. k úbytku hmyzu a tedy ke snížení potravní nabídky pro ještěrky). Dalším aspektem způsobu aplikace herbicidů může být negativní ovlivnění zoocenóz (včetně ještěrek) přímo chemickými látkami, které jsou jejich součástí.

Snížení atraktivity biotopu: V několika případech byl zaznamenán stav, kdy způsob údržby některých ploch nekoresponduje s ekologickými nároky ještěrek. Jako příklad lze uvést např. situaci zaznamenanou v rámci studijní plochy 5, která patřila v jarním období 2015 k částem lokality, kde byla

Pohled na zarůstající jižně exponovaný svah v rámci plochy 2 (27.4.2015)
View of the south exposed slope within area 2 with growing vegetation coverage (27.4.2015) Foto/Photo David Fischer

opakovaně zaznamenána druhá nejvyšší frekvence výskytu ještěrek (po ploše 1). V průběhu července byla proluka pečlivě pokosena a vyklizena, čímž došlo k rapidnímu poklesu atraktivity této plochy v důsledku snížení její úkrytové kapacity (ještěrky zde od tohoto termínu již nebyly vůbec pozorovány).

Na druhou stranu úbytek vhodných ploch pro ještěrky způsobuje i masivní zarůstání (absence managementu) potenciálně zajímavých biotopů hustým porostem keřů a dřevin (např. plochy navazující severovýchodně na plochu 4). Na místní populaci ještěrek může mít vliv i rozšiřování zastavěných ploch do biotopů, kde se tito živočichové vyskytují, popř. se vyskytovat za určitých podmínek mohou – např. plocha 4.

Jako příklad, kdy by naopak bylo možné využít potenciál dané části lokality pro ještěrky, lze zmínit nově sanované jižně exponované svahy navazující východně na plochu 1. V daném svahu byla v roce 2015 založena vinice, která – bude-li mít prvky umožňující osídlení této plochy xerofilní biotou – se může stát atraktivním domovem ještěrky zelené.

Negativním se naopak neukazuje překrytí části skalních výchozů ochrannou drátěnou sítí. Toto řešení (z pohledu bezpečného provozu zahrady nevyhnutelné) sice esteticky narušuje vnímání skalních biotopů, nicméně pro ještěrky představuje daný prvek zřejmě vítané rozšíření úkrytové kapacity stanoviště (byly opakovaně pozorovány, jak se pod drátěnou síť ukrývají).

Rušení ze strany návštěvníků a usmrcování jedinců pohybující se technikou: Jedná se o jevy, jejichž celkové dopady na místní populaci ještěrky zelené jsou s největší pravděpodobností zcela marginální. Ještěrky běžně využívají ke slunění plochy v bezprostřední blízkosti frekventovaných návštěvnických tras. Nelze vyloučit, že přítomnost návštěvníků, plynule proudících po vyhrazených plochách, by mohla mít i antipredační význam, a že ještěrky tuto okolnost pozitivně vnímají. Nebezpečí jejich usmrcování obslužnou technikou považujeme vzhledem k rychlosti pohybu za minimální (na rozdíl od jiných druhů, jako jsou např. obojživelníci nebo juvenilní stadia užovek podplamatých, jejichž usmrcování projíždějící technikou bylo v areálu zahrady potvrzeno). Nelze vyloučit náhodné usmrcení ještěrky při kosení porostů pomocí motorových sekaček či motorových kos (v roce 2015 bylo takto v ploše 4 zaznamenáno např. usmrcení užovky hladké).

Ještěrka zelená (označená šipkou) vyhřívající se poblíž informačního panelu na ploše č.5
European green lizard (indicated by the arrow) basking close to the information panel at the area No.5
Foto/Photo Petr Velenský“

Stěžejním prvkem stanovišť atraktivních pro výskyt ještěrek jsou úkryty v bezprostřední blízkosti vhodných míst ke slunění (nejatraktivnější jsou pak prvky, které tyto dva aspekty kombinují). V případě obnovy stepních svahů je tak třeba v první řadě pokud možno okamžitě při zásahu ponechávat mozaikovitý výskyt křovin (ideální pokryvnost cca 20% plochy). Důležité ale je, aby ponechané keře byly zavětveny co nejníže k terénu, jen tak mohou sloužit jako vhodný úkryt. V případě, že se na dané ploše takovéto keře nevyskytují, lze tohoto stavu dosáhnout podporou zmlazování vybraných jedinců (seříznutí bez aplikace herbicidu). Úkrytový potenciál stanoviště lze dále zvyšovat budováním skládaných zídek (ideálně jednou stranou zapuštěných do terénu) či ponecháním ležících kmenů. Jako velmi vhodné přechodné řešení (minimálně do obnovy žádoucího vegetačního krytu) lze využít ponechání roztroušených skupinek menších hromad větví.

Jednorázová velkoplošná likvidace veškerého porostu dřevin a křovin se jeví jako nepříliš vhodné řešení – tento postup lze zvolit za situace, kdy se jedná o stanoviště, kde se ještěrky již prokazatelně nevyskytují (případ sanace plochy 1). I tak je velmi důležité okamžitě „vybavit“ nově vzniklou plochu některými výše uvedenými prvky, zvyšujícími jeho heterogenitu a tak i atraktivitu pro ještěrky (opět případ plochy 1, kde se ukazuje, že ještěrky prioritně kolonizovaly buďto plochy, kde mohl být ponechán mozaikovitý kryt keřů nebo plochy, kde byly zbudovány výše uvedené prvky zvyšující úkrytovou kapacitu lokality).

Pokud dochází k úpravám ploch, kde se ještěrky aktuálně vyskytují (např. plocha 2), je důležité je provádět tak, aby tyto biotopy zůstaly pro ještěrky kontinuálně atraktivní (viz výše uvedené ponechání určitého procenta křovin, zbudování nových úkrytů a míst ke slunění atd.). Pokud situace vyžaduje razantnější zásah, je vhodné jej provádět etapovitě, nikoliv jednorázově na celé zájmové ploše.

Na studijních plochách obývaných ještěrkami lze doporučit pro zvýšení jejich atraktivity pro ještěrky následující:

Plocha 1: Většina této plochy není aktuálně ještěrkami prakticky vůbec využívána. Tuto situaci lze změnit zvýšením heterogenity daných ploch (= zvýšením úkrytové kapacity). V daném případě se v první řadě nabízí kombinace všech výše uvedených prvků – tj. zbudování většího počtu skládaných zídek částečně zapuštěných do terénu, instalace větších kusů kmenů do zcela odlesněných partií svahu, popř. dosazení keřů (ideálně např. šípky). Zejména v ploše 1 se pak nabízí využití značného množství pařezů pokácených stromů. Pokud by se podařilo pod nimi vytvořit dostatečně prostorné a hluboké dutiny, vznikla by zde řada nových vhodných úkrytů pro ještěrky.

Plocha 2 (včetně přilehlých částí jižně a jihozápadně orientovaného svahu až k hranici plochy 4): Zde je třeba provést citlivý managementový zásah s ponecháním mozaikovitého porostu nízkých, až k zemi zavětvených keřů (ideálně např. šípky). Tyto porosty je dále třeba v daném rozsahu udržovat (seříznutím a zmlazením dřevin lze dosáhnout postupně i žádoucí podoby (větve až k terénu)). V ploše lze ponechat na vhodných místech i menší kupky větví, popř. zaklíněné kmeny poražených stromů. Vzhledem k tomu, že se jedná o část lokality se stěžejním významem pro místní populaci užovky podplamaté, je třeba managementové zásahy sladit i s případnými specifickými stanovištními nároky tohoto druhu.

Případná aplikace herbicidů musí být prováděna lokálně (na řez, na list), nikoliv plošným postřikem.

Plocha 3: Plochu, minimálně několik metrů široký pás navazující na okraj cesty a proluky mezi voliérami, by bylo vhodné udržovat ve stavu, kdy zde nebude kompaktní porost keřové a stromové vegetace. Vhodné by bylo stávající vegetační kryt proředit na mozaiku, kdy by alespoň 50 % ploch bylo dobře osluněno a doplnit tuto část lokality o prvky, zvyšující atraktivitu pro ještěrky. V daném případě by se jednalo především o skládané zídky, popř. různé formace větších kamenů. Vhodné a ještěrkami využívané jsou i plochy částečně porostlé popínavou vegetací. Likvidovat je třeba porost škumpy.

Plocha 4: V jihovýchodní (ploché) části lokality by bylo vhodné proředit porost vzrostlých dřevin (cca na polovinu současného stavu) a lze sem umístit některé prvky zvyšující atraktivitu plochy pro ještěrky a plazy obecně (jedná se o velmi významnou část lokality pro užovky podplamaté). Kromě skládaných zídek, ponechaných kmenů či drobných kupek větví lze použít i volně nakupené hromady větších kamenů. V rámci pravidelně kosených ploch (např. kolem hřiště a vedle umístěné budovy) by bylo vhodné ponechávat alespoň přechodně nekosené (po dobu aktivity ještěrek) úzké lemy v jejich

Vhodně provedený management v rámci východní části plochy 1 (26.8.2015)
Properly executed management within the eastern part of area 1 (26.8.2015) Foto/Photo David Fischer

obvodu (pokoseny by byly až v pozdně podzimním období). Části lokality navazující na plochu 4 ze severozápadu (území mezi lokalitou 4 a 2) by bylo vhodné upravit stejně, jako je navrhováno v ploše 2 (ale s ohledem na význam z pohledu užovky podplamaté). Část se vzrostlými dřevinami ležící nejvýchodněji (nad zastavěnými plochami) lze ponechat v současném stavu.

V rámci případné výstavby nových objektů by měl být brán zřetel na význam dané plochy (architektonické řešení staveb a jejich okolí, podpůrná a kompenzační opatření pro plazy, omezení výstavby v nejcennějších plochách).

Plocha 5: Pro ještěrky zelené velmi významná část lokality (po ploše 1 zde byla zaznamenána nejvyšší frekvence výskytu). Pravděpodobně mimořádně důležitý (a jediný) migrační koridor spojující výše položené části lokality se stanovišti v dolní části zahrady (např. kolem vodního kanálu) – zbytek svahu totiž zabírají venkovní výběhy a expozice, které zřejmě nejsou ještěrkami využívány.

Tato plocha byla první, na které byla v roce 2016 realizována opatření, vycházející z odborné expertízy (Fischer 2015). Výrazné obohacení prostoru skládanými valy a zídkami, přítomnost menších vodních ploch a ponechání hustého vegetačního krytu lučními rostlinami přineslo v roce 2017 návrat a posílení místní populace ještěrek. Při letmém průzkumu 22.-23.7. 2017 zde bylo pozorováno nejméně 6 ještěrek zelených (dva samci, dvě samice a dva subadultní jedinci).

Z pohledu místní populace ještěrky zelené je mimořádně důležité, aby tato plocha zůstala zachována ve stavu vyhovujícím pro ještěrky – včetně zachování dostatečně hustého, disperzně kvetoucího, vegetačního pokryvu.

Plocha 6: V rámci dané plochy by bylo vhodné zbudovat v osluněných partiích svahu další kamenné zídky. Jinak je třeba lokalitu udržovat zhruba v současné podobě (důležité je zachování osluněných ploch).

Velkou výzvou zůstává nově odlesněný svah, na kterém byla v roce 2015 vybudována vinice, pod níž je budován Rákosův pavilón. Vhodnými úpravami by bylo možno dosáhnout výrazného zvýšení atraktivity této plochy pro ještěrky. Inspirací přitom mohou být např. stávající vinice v Podyjí (Šobes), které optimálně propojují produkci vinné révy s biologickou funkcí tohoto stanoviště – jedná se o lokalitu s nesmírně početnou populací ještěrky zelené a dalších vzácných druhů plazů, jako je např. užovka podplamatá a užovka stromová. Všechny tyto druhy jsou zde vázány do značné míry na skládané zídky,

Skládaná zídka s vysokou atraktivitou pro ještěrky – plocha 6 (26.8.2015)
Wall composed of stacked stones highly attractive for lizards - Area 6 (26.8.2015) Foto/Photo David Fischer

které tvoří oporu jednotlivých teras (podobná zídka je v nově zbudované vinici v zoo při jejím dolním okraji), popř. na okrajové partie vinic stepního charakteru. Optimálním řešením by tak bylo doplnění těchto, popř. dalších prvků zvyšujících heterogenitu lokality, i do nové vinice na území Zoo Praha. Je velmi pravděpodobné, že by se z této části zahrady stal nesmírně atraktivní biotop pro místní druhy plazů, včetně ještěrky zelené. K prosperitě xerotermních organismů by přispěla i minimalizace případného použití biocidů a jiných potenciálně nebezpečných látek.

Předmětem dalšího podrobného výzkumu (instalace fotopastí atd.) by měl být predační tlak (nejen ze strany koček) a jeho dopad na populaci. V areálu Zoo Praha by bylo vhodné eliminovat volně se pohybující kočky. Rovněž je potřebné sledovat vliv migračních bariér a eventuálně hledat možnosti migračního zprůchodnění „Zakázanky".

Používání herbicidů by mělo být omezeno na nutné minimum a mělo by docházet pouze k cílené lokální aplikaci – plošná aplikace postřikem je nežádoucí. Dále by bylo velmi vhodné ověřit složení a možné dopady (včetně těch způsobených dlouhodobou kumulací některých látek) používaných herbicidů na ještěrky, do jejichž organismu se mohou dostávat jak přímým kontaktem, tak např. při přijímání tekutin nebo druhotně s potravou (hmyzem který např. pozřel ošetřenou vegetaci). Velmi vhodné by bylo, vzhledem ke skutečnosti, že herbicidy jsou na území Zoo Praha používány poměrně běžně, analyzovat dostupnou literaturu zabývající se problematikou vlivu herbicidů na různé skupiny živočichů.

Údržba ploch obývaných ještěrkami a pro ještěrky potenciálně vhodných, by tam, kde je to možné v kombinaci s provozními potřebami Zoo Praha, měla respektovat jejich ekologické nároky (ponechání enkláv s vyšší vegetací, zachování heterogenity porostů, dostatečné oslunění, kosení s ohledem na minimalizaci nebezpečí usmrcování ještěrek).

Při plánování staveb by měl být dle možností brán ohled na výskyt ještěrek (a dalších zvláště chráněných druhů rostlin a živočichů) a veškeré plochy s potvrzeným výskytem ještěrek by měly být v co nejvyšší možné míře chráněny před poškozováním. V rámci současných lokalit s potvrzeným výskytem ještěrek má zásadní význam zachování plochy 5 – jedná se zjevně o velmi významný migrační koridor.

Poděkování za cenné poznatky k výskytu ještěrek zelených v Zoo Praha patří panu Vojtěchu Vítovi, zaměstnanci Zoo Praha.

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The European green lizard, *Lacerta viridis*, is in the Czech Republic listed as a critically endangered species by the local legislative regulations. Populations in the Bohemia region are located beyond the northern border of continuous range of the species and are closely related to the so-called river phenomenon of deeply engorged river valleys. This phenomenon describes specific microclimatic conditions which allow thermophilous species to survive outside of their continuous range. The distribution of the Bohemian populations is the result of post-glacial expansion of the species from south-glacial refuges and these populations are geographically and genetically isolated from each other.

The grounds of Prague zoo are located in such area with distinctive microclimate and a slope with south and west exposition, suitable for inhabitation by several reptile species. Moreover, due to the specific nature of the space usage and construction, the lizard population can survive in large parts of the zoo area even though the space is used for different purpose. The *Lacerta viridis* population located on the grounds of Prague zoo, as an isolated autochtonnous relict population, has a significant conservation value due to its genetic distinction and requires specific management that considers low abundance, habitat fragmentation, low genetic variability and isolation as some of the the most threatening factors.

Even though some management measures were applied earlier, the intensive conservation management took place in the Prague zoo in the period from 2011 to 2015 with the focus on renewing the rocky steppe biotope in the area of Zakázanka. Several smaller interventions were made in the years 2016 and 2017, mostly in smaller areas or in maintaining parts of the area which was a subject to earlier management. The recent paper contains a critical evaluation of these management measures and proposes additional.

The population has been continuously monitored by the authors of the recent paper and Zoo employees. The intensive field research was carried out in 2015 in the south and southwest slope of the Vltava river. The *Lacerta viridis* individuals were not marked, so the abundance is estimated from line transects. In total, seven study areas were chosen (see Map 1).

The individuals were found in each of the chosen study area at least once, with the most findings (52,1 %) in area 1, area 5 contained 29,6 % of findings. Other areas contained from 1,4 % to 5,6 % findings (see Table 2). Before the management measures, the findings were concentrated in areas 1, 2 and 4. The numbers of findings between study areas are highly unbalanced and even within the individual study areas, lizards use only their parts. That consists with data from close populations of *Lacerta viridis* with the availability of shelter and basking places as a decisive parameter.

The number of demonstrably different individuals we have observed is 30. Based on the presumption that not all areas suitable for *Lacerta viridis* were monitored, the season of 2015 was not typical in terms of climate conditions (long dry periods) and there were just 7 full day visits at the site, the qualified estimation of the abundance is approximately at least twice this number (60).

The observed age composition of the population based only on demonstrably different individuals during one visit is 1 : 0.12 : 0.65 (adults : subadults : juveniles), based on all findings 1 : 0.16 : 0.45.

Sex ratio was observed from demonstrably different individuals is 0.88 : 1 (M : F) and 1.33 : 1 when based on all findings.

Based on the total area of 2 ha, the observed density is approximately 15 individuals / ha. The authors estimate the real total density to be higher as they do with abundance.

The number of individuals with regenerated tail was very low (6,7 % of demonstrably different individuals and 4,2 % from all findings). The closest population of *Lacerta viridis* in Tiché údolí contained 26 % individuals with regenerated tail.

Even though the management measures definitely raised attractiveness of large parts of Prague zoo area for *Lacerta viridis* and allowed spreading to new habitats, some aspects of presence on the grounds of Prague zoo already have or could have potentially negative impact on the local population.

In some parts of the area inhabited by lizards is a low availability of shelters. Especially in study areas 1 and 2 where some parts have been completely devoid of bushes and left temporarily unusable for the lizards. This destroys not only potential shelters, but also makes the whole area exposed to direct sunlight and prevents thermoregulation in hot weather. This measure was necessary in areas that had to be rid of trunk acacia which covered them continuously and there was no other vegetation present to be left on site. The lack of shelter was partially compensated by leaving some of the cut branches or smaller trunks on site. Also, the usage of herbicides (Roundup) could affect the lizard population either directly or indirectly through the food chain.

Other negative aspect of presence on the grounds of Prague zoo could be a concrete curbs of pathway in the area of Zakázanka, which can disrupt a possibly important migration corridor for *Lacerta viridis*.

The Prague zoo is also a home to several freely living potential predators. A large number of freely roaming cats has been observed. Only predator with directly observed predation on *Lacerta viridis* present on site is Kestrel (*Falco tinnunculus)*. Indirect observation show also predation by European magpie *(Pica pica)*. The potential predators include: Smooth snake *(Coronella austriaca)* even though their abundance seems to be extremely low, Common pheasant (*Phasianus colchicus)*, Grey heron (*Ardea cinerea)*, freely roaming Indian peafowl *(Pavo cristatus),* other possible but probably not with a large impact on the population could be rats, hedgehogs and foxes. Even though this predation can be considered natural (except cats, peafowls and rats), it can pose a serious threat to a population with such a low abundance.

The management measures could have lowered attractivity of some parts of the area by too intensive vegetation removal. For example, part of study area 5 which was previously used by lizards was not temporarily used after the management measures in July 2015. The opposite could be newly constructed vineyard which connects to study area 1 and is expected to provide a new and attractive habitat. A new hand-laid stone wall with sufficient inner spaces was constructed in area 5 and was immediately settled by lizards. Also, the slope stabilization by steel mesh which was installed on parts of the slope proved to increase the attractivity of the biotope and the mesh is used by the lizards as a temporary shelter.

The effect of a large number of visitors could be predicted to be significantly negative, but the observations show otherwise. Lizards use basking places in close proximity to pathways and corridors with visitors (it is not excluded that lizards recognize the antipredatory value of visitors´ presence) and react only if a human cross the border of the corridor.

Based on the observations and evaluation of previous management measures, recent paper proposes several new measures and modification of ongoing ones.

The most important recommendation is to leave parts of the vegetation on site and retain the landscape mosaic (with the ideal vegetation coverage of 20 %). The bushes left on site should have branches close to surface to provide shelter. The availability of shelters can also be raised by leaving cut branches on site in small piles.

Heterogenity of areas completely rid of vegetation should be raised by constructing stone walls, installation of solitary stones, temporary placement of piles of branches and planting new bushes in sufficient distances. These measures should also be considered in the new vineyard, especially stone walls which could either create terraces (one is already present at the bottom of the vineyard), or line the edges.

The next part of research should be focused on predation (for example by photo traps) and its impact on the population. Also, the impact of possible migration barriers should be thoroughly examined.

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Distribution of the European green lizard, *Lacerta viridis* (Squamata: Lacertidae), in the Czech Republic: Real data and a predictive model

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Abstract. The European green lizard, *Lacerta viridis* (Laurenti, 1768), is listed in the Czech Republic as critically endangered. Its distribution in the Bohemia region is restricted to small isolated local populations which are located beyond the northern border of continuous range of this species and are closely related to very specific habitats. Populations in southern Moravia form the northwestern boundary of the continuous distribution of the species. Based on the statewide database of the Czech Nature Conservation Agency, we created a predictive model and determined key factors influencing the species distribution in the Czech Republic. The most relevant factors were: annual precipitation, terrain slope, average temperature of the warmest quarter and precipitation in the coldest quarter. The model is well compatible with published data on *Lacerta viridis* distribution in the Czech Republic and is applicable in both theory and practice of the species conservation – e.g. focusing faunistic research to certain areas, critical analysis of controversial presence reports and as an input for species management in the form of repatriation and introduction.

Key words. Distribution, predictive model, species management, conservation, monitoring, river phenomenon.

INTRODUCTION

In ecology, predictive models are becoming increasingly popular as a tool for complex distribution analysis and identification of key climatic and geographical factors (Elith et al. 2006, Civiš 2013). As the number of studies increases, the focus is not only on the distribution of plants, but is expanding to animals, including reptiles (Kaliontzopoulou et al. 2008, Sillero & Carretero 2013, Oraie et al. 2014, Hosseinian Yousefkhani et al. 2015, Wirga & Majtyka 2015), even in a relation to climate change (Dubey et al. 2013).

In the Czech Republic, the European green lizard *Lacerta viridis* (Laurenti, 1768) is generally rare and declining, as a result of habitat degradation (Baruš et al. 1989, Rehák 1996, 2015, 2017, Mikátová & Nečas 1997, Moravec 2015). According to legislative regulations in the Czech Republic, the European green lizard remains listed among the critically endangered species even though the current Red list for the Czech Republic decreased the category to endangered (Jeřábková et al. 2017). The reason for this change is a generally better state of populations in southern Moravia (in contrast to populations in Bohemia).

Although the oldest (Lower Miocene) central European fossil remnants of a lizard closely related to *Lacerta viridis* have been described from Dolnice in the Cheb District (Čerňanský 2010), the recent distribution of European green lizard in the Czech Republic is the result of post-glacial expansion of the species from south-glacial refuges (Baruš et al. 1992, Godinho et al. 2005, Böhme et al. 2006, Rehák 2015). All Czech populations belong to the nominotypical subspecies. In Bohemia, the European green lizard distribution is isolated from the continuous range of the species (Nettmann & Rykena 1984, Gasc et al. 1997, Crnobrnja-Isailović 2009, Moravec 2015, Rehák 2015).

Molecular data confirmed genetic affinities of the Bohemian populations to those in neighboring parts of *Lacerta viridis* distribution in NE Germany (Elbe River) and Moravia (Böhme et al. 2006, Böhme & Moravec 2011). Moreover, individual relic Bohemian populations are also more or less isolated from each other and genetically slightly distinct (Böhme et al. 2006, 2007, Böhme & Moravec 2011). These populations are ecologically notable as inhabitants of biotopes retaining ancient characteristics and they can differ significantly in autecology parameters (Fischer & Rehák 2010, Blažek 2013, Chmelař 2014, Rehák 2015, Fischer et al. 2016).

Since 2007, Czech Nature Conservation Agency has been monitoring presence of the European green lizard in order to get the most up-to-date and comprehensive picture of the species distribution. While certain places of occurrence have traditionally been well known in the long term since 1851 (Štěpánek 1949, Moravec 2015), no published data are available in other areas of the Czech Republic, where natural conditions do not exclude the presence of *Lacerta viridis*.

The focus of recent paper is to review yet unpublished Nature Conservation Agency faunistic reports, to analyze available distribution records, to identify the key factors affecting the distribution of European green lizards and to create a predictive model of the European green lizard distribution in the Czech Republic. We intend to help to prioritize the monitoring effort (to focus on places where the predicted probability of presence is high, but no real presence has been recorded). At the same time – by comparing the predictive model with the known distribution of the European green lizard in the Czech Republic (based on critically evaluated published and our own data) – to evaluate the usefulness of creating predictive distribution models for the theory and practice of conservation and species management in particular.

MATERIAL AND METHODS

As a source of the European green lizard presence sites was used a statewide database maintained by the Nature Conservation Agency of the Czech Republic. The database contained 1,333 records from the whole territory of the Czech Republic collected within the period from 1932 to 2014, with only 103 records being older than 1980.

The Predictive Distribution Model was created using MaxEnt software, version 3.3.3k, the output being a GIS document in .asc format. This software was specifically chosen to work well with presence-only data (Elith et al. 2006, Hernandez et al. 2006). As the European green lizard presence prediction value, the "Logistic threshold" was defined, i.e. the optimization between the sensitivity of the model and the location of all real places of presence in the predicted areas.

For modeling purposes, a total of 74 layers were created for the Czech Republic: the lowest, highest and average temperatures for individual months (36 layers in total), precipitation in individual months (12), bioclimatic variables according to worldclim.org methodology – see Appendix (19), altitude, surface exposure, human footprint, slope, road network, water bodies, watercourses (Civiš 2013).

For modeling via the Maxent interface, WorldClim Worldwide database is standardly used as the source of climate variables. This database, however, uses only data from two meteorological stations for the Czech Republic (Hijmans et al. 2005), which is why it was inappropriate for our research, and we have created layers for the bioclimatic variables manually.

All layers were created in ArcGis 9.3 (ESRI 2008) in the 2D coordinate system S-JTSK Krovak East-North. The layers of climate variables were created based on the Climate Atlas of Czechia (Tolasz et al. 2007), which includes data from 1961 to 2000.

RESULTS AND DISCUSSION

The predictive strength of the model is very high (AUC=0.958). Only 4.2% of the actual real presence points were located below the predicted occurrence threshold, including several remote presence points from areas where the European green lizard occurrence is unlikely – for example area near Krnov and Mladá Boleslav. These are points that the national database has taken over from sources labeled as controversial. The number of these points is negligible and we consider it a tax for a large data sample from a wide spectrum of informants. Since the database is only open for authorized zoologists, included records are reliable and the size of the input data set contributes to prediction strength (Hernandez et al. 2006, Merow et al. 2013). The "logistic threshold" prediction, when the sensitivity of the model is equal to its specificity, was 0.208. Therefore, any higher value means that the model predicts the European green lizard presence, see Fig. 1. The most important contributors to the resulting model were: BIO 12 – annual precipitation (explaining 29.9% variability), slope – terrain slope (12.9%), BIO 10 – Average temperature of the warmest quarter (10.5%) and BIO 19 – precipitation in the coldest quarter (9.2%). Graphs of these variables are shown in Figs. 2–5.

According to the model, the probability of presence of the European green lizards is highest in the range of annual precipitation between 500–550 mm (Fig. 2). The probability also grows with increasing inclination of the terrain up to 20–25° (Fig. 3). It is almost constant at the average temperatures of the warmest quarter 10–18 °C, the highest for temperatures of 19–20 °C and above 20 °C sharply drops below the threshold of prediction (Fig. 4). According to the precipitation in the coldest quarter, the probability is highest if it exceeds 150 mm (Fig. 5).

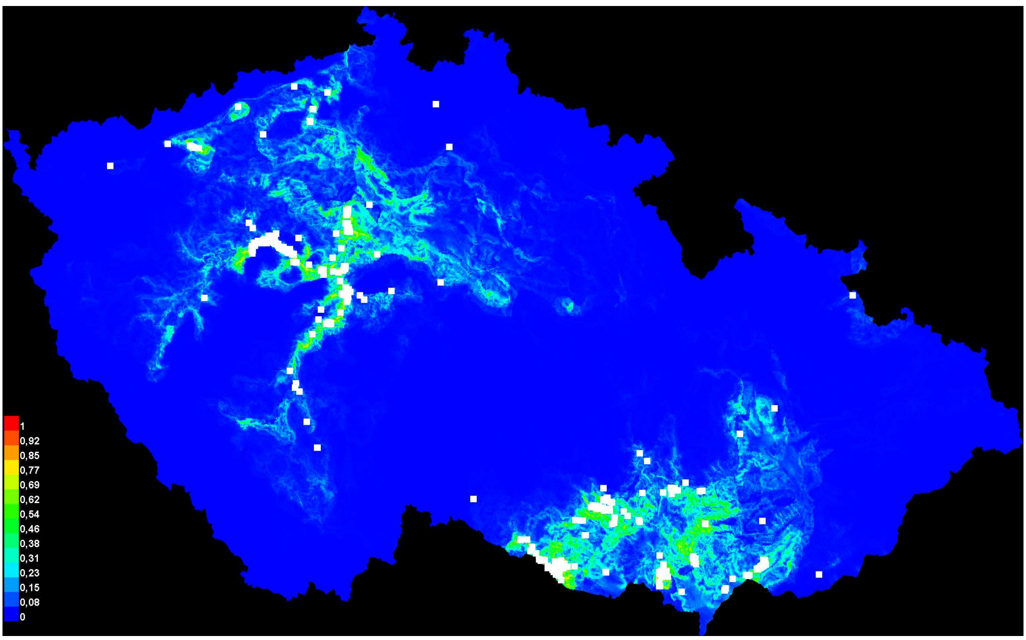

Fig. 1. Predicted distribution of the European green lizard, *Lacerta viridis* (Laurenti, 1768) in the Czech Republic – white points represent recorded presences from the national database.

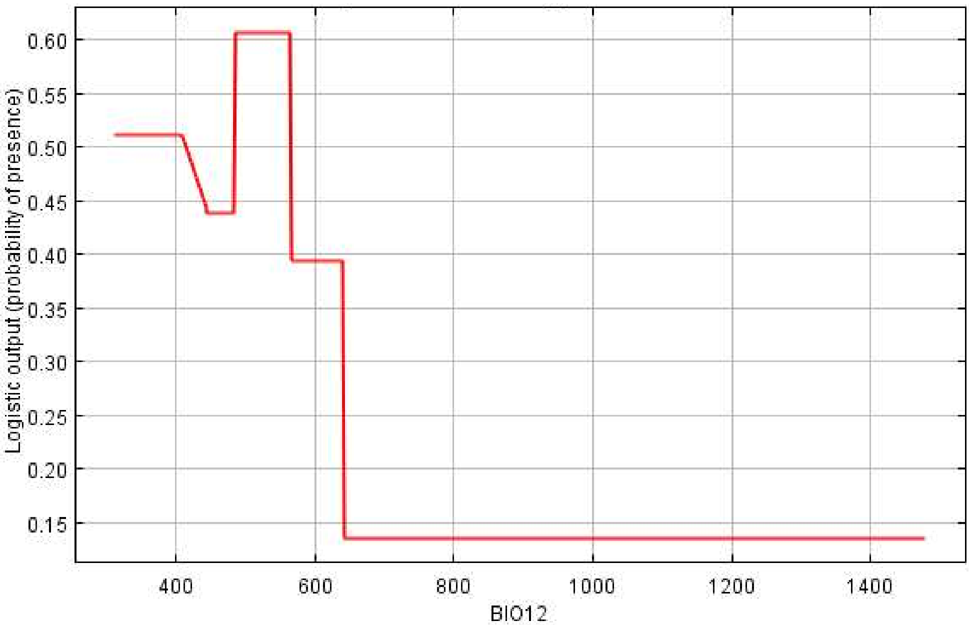

Fig. 2. Response curve of the annual precipitation variable (mm).

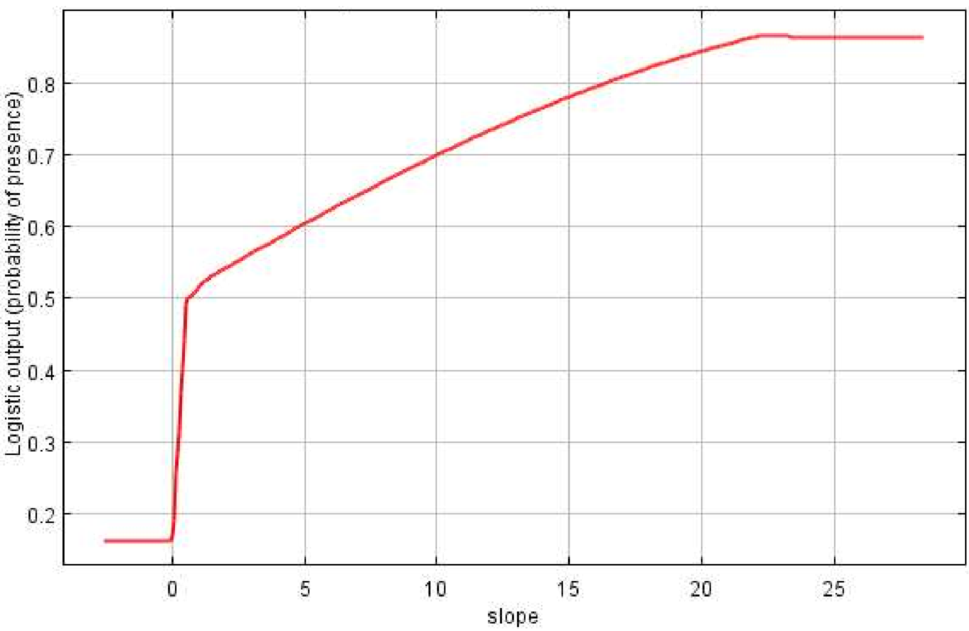

Fig. 3. Response curve of the slope inclination variable (°).

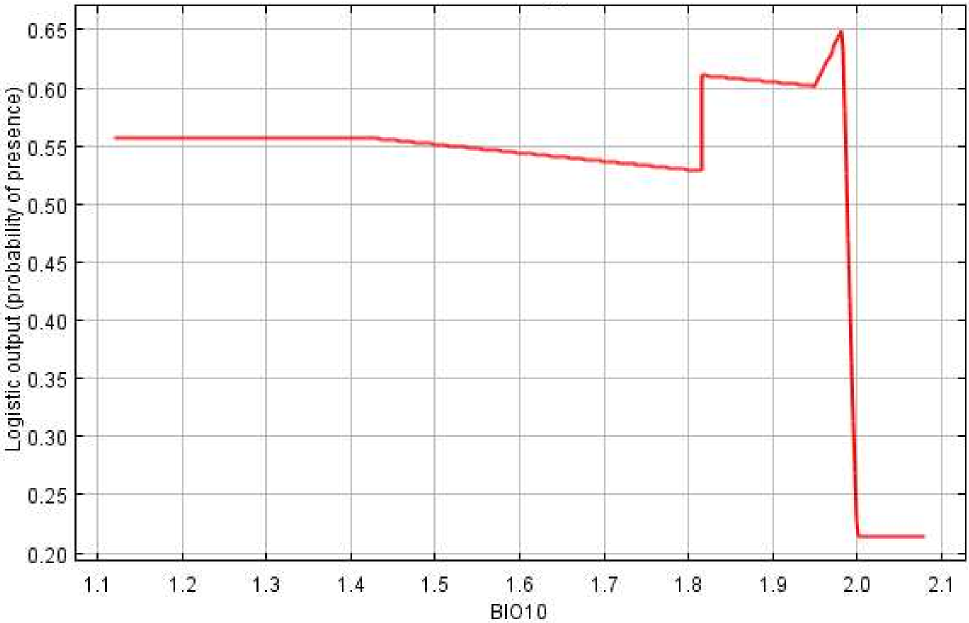

Fig. 4. Response curve of the mean temperature of warmest quarter variable (°C / 10).

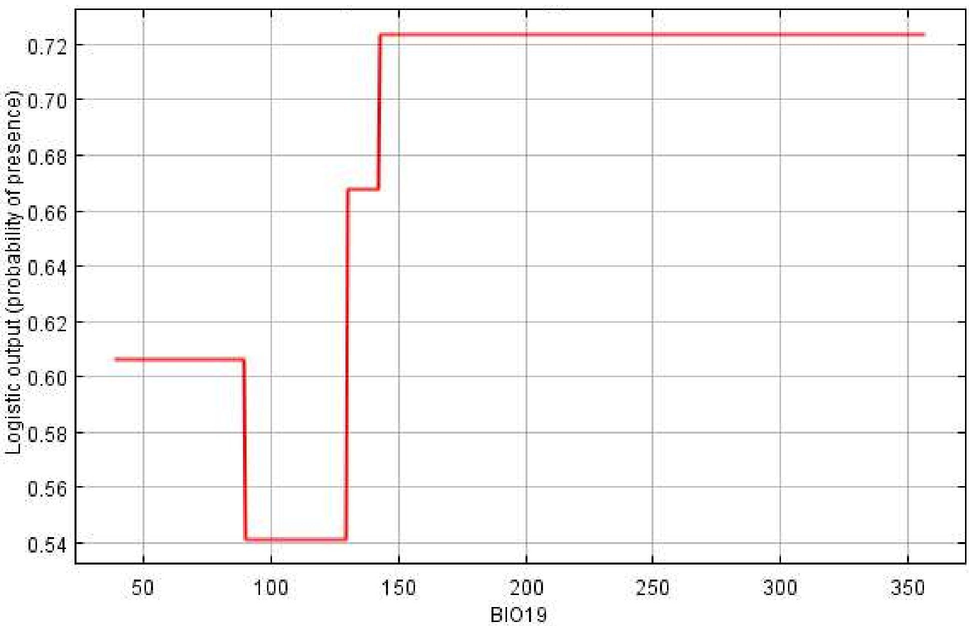

Fig. 5. Response curve of precipitation in coldest quarter variable (mm).

The annual precipitation variable is an expected result, given that the green lizards are located mostly in warm and dry habitats in Central Europe. The precipitation range 500–550 mm per year corresponds to the avoidance of excessively humid locations (Mikátová 2001, 2002, Fischer & Rehák 2010, Moravec 2015).

The increasing probability of presence with increasing slope inclination corresponds to the theory of *Lacerta viridis* distribution being linked to so-called river phenomenon (Jeník & Slavíková 1964, Vannote et al. 1980, Ložek 1988, Strödicke 1995, Ward 1998, Mikátová 2001). This theory indicates that thermophilous organisms can be found behind the northern boundary of their continuous area in the deeply cut river valleys on the slopes with southern exposition. For the European green lizard distribution in Bohemia, the link to the river phenomenon was well documented (Ložek 1988, Mikátová et al. 2001, Mikátová 2002, Fischer & Rehák 2010, Moravec 2015, Rehák 2015, Fischer et al. 2016). The resulting map of the species presence in Bohemia is in full compliance with the occurrence of a river phenomenon in the Czech Republic. In this model, the slope explains a large portion of variability, but only when combined with other significant variables. Examples of European green lizards from localities in Bohemia (isolated from the continuous area of species distribution) and from localities in South Moravia (northwestern border of the continuous species range) are shown in Figs. 6–12.

The dependence of prediction on average temperatures in the warmest quarter is self-evident, but it is interesting to see a sharp drop when exceeding the 20 °C threshold. During sunny weather,

Fig. 6. *Lacerta viridis* (Laurenti, 1768) – adult male, Praha-Troja, Central Bohemia, 27 April 2018. Photo by I. Rehák.

Fig. 7. *Lacerta viridis* (Laurenti, 1768) – adult female, Albertovy skály, Central Bohemia, 28 May 2016. Photo by D. Fischer.

the surface temperatures of the exposed parts of habitats can easily exceed 80 °C, it is likely that the long-term excess of these temperatures will have a negative effect on the green lizard presence. There is also a strong assumption of intercorrelation of average annual precipitation with average annual temperatures (Schulz & Halpert 1993).

Dependence on precipitation in the coldest quarter is somewhat surprising. Based on this, the probability of presence is growing. A possible interpretation is that the European green lizards require a snow cover for a successful wintering. The snow isolates the wintering site from extreme frosting, but also from sun heating, which can interrupt wintering, and an individual will often not survive this interruption in hibernation (Vongrej et al. 2007).

The most recently published map of distribution (Moravec 2015) is in good consistency with our predictive model, but since it is a square network map without precise locations and coordinates, a more detailed comparison is problematic – especially with regard to the extraordinarily variable geomorphological relief of the Czech Republic and to the distribution of the European green lizard in the Czech Republic, where there is a strong correlation with the geomorphological relief variability – in this case the network mapping easily includes the areas where the species is not present.

We consider the result of finding large areas with high probability of modeled prediction where real occurrence is not reported to be extraordinarily important. Here we see the need to direct species monitoring to these places and search for historical data. Also, these sites should be considered as a matter of priority for possible repatriation efforts and for conservation management by species introduction if the nature conservation authority decides for them.

The comparison of our predictive model and real distribution shows that the predicted and real distribution is almost fully accordant. Thus we consider a creation of predictive distribution models as a helpful instrument to facilitate monitoring and conservation efforts.

Fig. 8. *Lacerta viridis* (Laurenti, 1768) – adult male, Havraníky, Southern Moravia, 5 June 2017. Photo by D. Fischer.

Fig. 9. *Lacerta viridis* (Laurenti, 1768) – adult female, Nový Hrádek, Southern Moravia, 26 May 2012. Photo by D. Fischer.

Fig. 10. *Lacerta viridis* (Laurenti, 1768) – adult male, Praha-Troja, Central Bohemia, 31 August 2020. Photo by I. Rehák.

Fig. 11. *Lacerta viridis* (Laurenti, 1768) – adult male, Šobes, Southern Moravia, 25 May 2015. Photo by D. Fischer.

Fig. 12. *Lacerta viridis* (Laurenti, 1768) – a couple (female on top), Albertovy skály, Central Bohemia, 17 May 2006. Photo by D. Fischer.

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APPENDIX

Bioclimatic variables according to worldclim.org methodology

BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp – min temp))
BIO3 = Isothermality (BIO2/BIO7) (×100)
BIO4 = Temperature Seasonality (standard deviation ×100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (BIO5-BIO6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter

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Research Article

Protecting isolated reptile populations outside their main area of distribution: a predictive model of the Dice snake, *Natrix tessellata*, distribution in the Czech Republic

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Abstract

Marginal populations of animals are highly susceptible to environmental pressures associated with climatic changes. Understanding their distribution and ecological requirements is, thus, essential for the development of efficient conservation strategies. The dice snake, *Natrix tessellata*, is listed as critically endangered in the Czech Republic. In certain regions (Bohemia and Silesia), its populations are located beyond the northern border of the continuous range of the species, while the south Moravian populations are connected to it. Based on the statewide database of the Czech Nature Conservation Agency, we created a predictive model and determined key factors influencing the species distribution. The most relevant factors were: watercourses and bodies, average annual temperatures, altitude, slope inclination and precipitation seasonality. The model fits the

presence records well and is applicable in both theory and practice of the species conservation – for example, focusing faunistic research to certain areas, critical analysis of controversial presence reports and as an input for species management in the form of repatriation and introduction.

Keywords

conservation, monitoring, species management, river phenomenon

Introduction

In ecology, predictive models are becoming increasingly popular as a tool for complex distribution analysis and identification of key climatic and geographical factors (Elith et al. 2006, Civiš 2013). As the number of studies increases, the focus is not only on the distribution of plants, but is expanding to animals, including reptiles (Kaliontzopoulou et al. 2008, Hosseinian Yousefkhani et al. 2013, Sillero and Carretero 2013, Oraie et al. 2014, Wirga and Majtyka 2015, Vargas-Ramírez et al. 2016, Petrosyan et al. 2020, Chmelař et al. 2020, Srinivasulu et al. 2021), predictions of invasive species spreading (Pyron et al. 2008, Jarnevich et al. 2018) and even in relation to climate change (Dubey et al. 2012). Especially small isolated populations are presumed to be most threatened by habitat erosion due to the climate change (Sinervo et al. 2010).

In the Czech Republic, the dice snake, *Natrix tessellata* (Laurenti, 1768) is generally rare and declining, as a result of habitat degradation (Pecina 1991, Velenský et al. 2011) and introduction of the invasive American mink (*Neovison vison*) that predates on *N. tessellata* (Kapler 1994, Mikátová et al. 2001, Šváb 2003, Musilová and Zavadil 2011). The habitat fragmentation due to roads and cycle paths that often lead close to the river banks and either degrade the habitat directly or block the migration route between feeding and wintering and reproduction sites are also factors. (Telenchev et al. 2017). According to legislative regulations in the Czech Republic, *N. tessellata* is listed amongst the critically endangered species according to the Ministry of the Environment of the Czech Republic and the current Red List of amphibians and reptiles for the Czech Republic lists the species as endangered (Jeřábková et al. 2017).

The recent distribution of *N. tessellata* in the Czech Republic is the result of post-glacial expansion of the species from south-glacial refuges, while the isolated Bohemian and German populations are presumed to be from Holocene climatic optimum (Guicking and Joger 2011); expansion of *N. tessellata* from glacial refuges was also documented for Asian populations (Jablonski et al. 2023). The origin and distribution in the Czech Republic are similar to the distribution of the European green lizard (*Lacerta viridis*) and the distribution of these species significantly overlaps mostly in river valleys in Bohemia, but also in southern parts of Moravia (Moravec 2015, Chmelař et al. 2020). In Bohemia, Silesia and Germany, *N. tessellata* distribution is isolated from the continuous range of the species and these individual populations are mostly isolated from each other (Gruschwitz and

Günther 1996, Mikátová et al. 2001, Vlček et al. 2010, Guicking and Joger 2011, Moravec 2015). Molecular data confirmed genetic affinities of Bohemian populations (samples from rivers Berounka and Ohře) to those in neighbouring parts of *N. tessellata* distribution in Germany, Bulgaria, Romanian Donau Delta, Slovakia and Serbia (Guicking et al. 2009). On the other hand, samples from the population near Havířov show a similar haplotype to populations in Hungary, southern Austria and Slovenia (Jablonski et al. 2014). Furthemore, recent molecular data confirm interspecific hybridisation within the genus *Natrix* (Asztalos et al. 2021, Schöneberg et al. 2023).

Since 2007, the Czech Nature Conservation Agency has been monitoring the presence of *N. tessellata* in order to obtain the most up-to-date and comprehensive picture of the species distribution. While certain places of occurrence have traditionally been well known in the long term since 1790 (Lindaker 1790, Štěpánek 1949, Moravec 2015), no published data are available in other areas of the Czech Republic, where natural conditions do not exclude the presence of *N. tessellata*.

The focus of this recent paper is to review yet unpublished Nature Conservation Agency faunistic reports, to analyse available distribution records, to identify the key factors affecting the distribution of *N. tessellata* and to create a predictive model of the species distribution in the Czech Republic. We intend to help to prioritise the monitoring effort (to focus on places where the predicted probability of presence is high, but no real presence has been recorded). At the same time – by comparing the predictive model with the known distribution of *N. tessellata* in the Czech Republic (based on critically evaluated published and our own data) – to evaluate the usefulness of creating predictive distribution models for the theory and practice of conservation and species management in particular.

Material and methods

As a source of *N. tessellata* presence sites, we used a statewide database maintained by the Nature Conservation Agency of the Czech Republic. The database contained 660 records from the whole territory of the Czech Republic collected within the period from 1895 to 2014, with only 102 records being older than 1980.

For modelling purposes, a total of 73 layers were created for the Czech Republic: the lowest, highest and average temperatures for individual months (36 layers in total), precipitation in individual months (12), bioclimatic variables according to worldclim.org methodology (Table 1) (19), altitude, surface exposure, human footprint, slope, road network, watercourses and -bodies (including a 200 m buffer on each side of the watercourse or -body) (Civiš 2013).

For modelling via the MaxEnt interface, WorldClim Worldwide database is routinely used as the source of climate variables. However, this database uses data from only two meteorological stations for the whole Czech Republic (Hijmans et al. 2005), which is why it was unsuitable for our research and we have created layers for the bioclimatic variables manually.

Table 1.
Bioclimatic variables according to worldclim.org methodology.

Variable	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

All layers were created in ArcGis 9.3 (ESRI ArcGIS 2008) in the 2D coordinate system S-JTSK Krovak East-North. The layers of climate variables were created, based on the Climate Atlas of Czechia (Czech Hydrometeorological Institute 2007), which includes data from 1961 to 2000.

Variables containing maximum, minumum and average temperatures and precipitation in individual months were not included in the model because of their high intercorrelation. In similar cases, a careful interpretation is recommended for making possible implications for species conservation (Syfert et al. 2013). These data were also already included in the WorldClim bioclimatic variables.

The climatic variables were screened for intercorrelation in ENM Tools (Warren et al. 2010), resulting in correlation matrices for the Pearson correlation coefficient "r", Pearson coefficient of determination "r^2" and Variance Inflation Factor "VIF" (Suppl. material 1). Variables with r > 0.8, r > 0.8 and VIF > 10 were considered heavily intercorrelated (Pradhan 2016) and removed from the model not to be used alongside variables with which they were closely correlated. The final model included the following variables: altitude, aspect, human footprint, road network, BIO 1, BIO 2, BIO 3, BIO 4, BIO 6, BIO 7, BIO 8, BIO 9, BIO 12, BIO 15, slope, watercourses and -bodies.

The Predictive Distribution Model was created using MaxEnt software (Phillips et al. 2023), the output being a GIS document in .asc format. This software was specifically chosen to work well with presence-only data (Elith et al. 2006, Hernandez et al. 2006). As the *N. tessellata* presence prediction value, the “Logistic threshold” was defined, i.e. the optimisation between the sensitivity of the model and the location of all real places of presence in the predicted areas. MaxEnt model was run in three replications and automatically cross-validated. All other settings in the model were set to default.

Results

The predictive strength of the model (mean AUC) was 0.92 (92% of actual presence records were above the prediction threshold) (Suppl. material 2). The mean “logistic threshold” prediction, when the sensitivity of the model is equal to its specificity, was 0.151. Therefore, any higher value means that the model predicts *N. tessellata* presence (see Fig. 1). The most important contributors to the resulting model were: Watercourses with 200 m buffer - Water_buffer (explaining 36.7% variability), BIO 1 – Average annual temperature (18.7%), altitude (11.6%), slope (11.4%) and BIO 15 - Precipitation seasonality (9.6%) Figs 2, 3, 4, 5, 6.

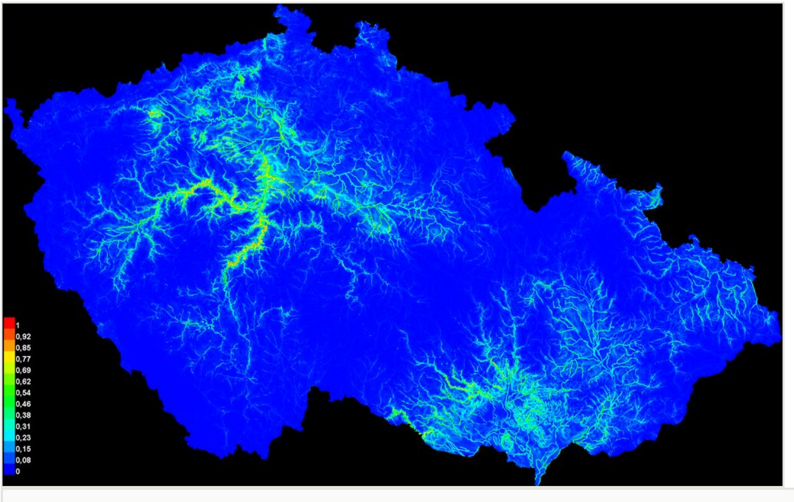

Figure 1. doi

Predicted distribution of the dice snake, *Natrix tessellata* (Laurenti, 1768), in the Czech Republic.

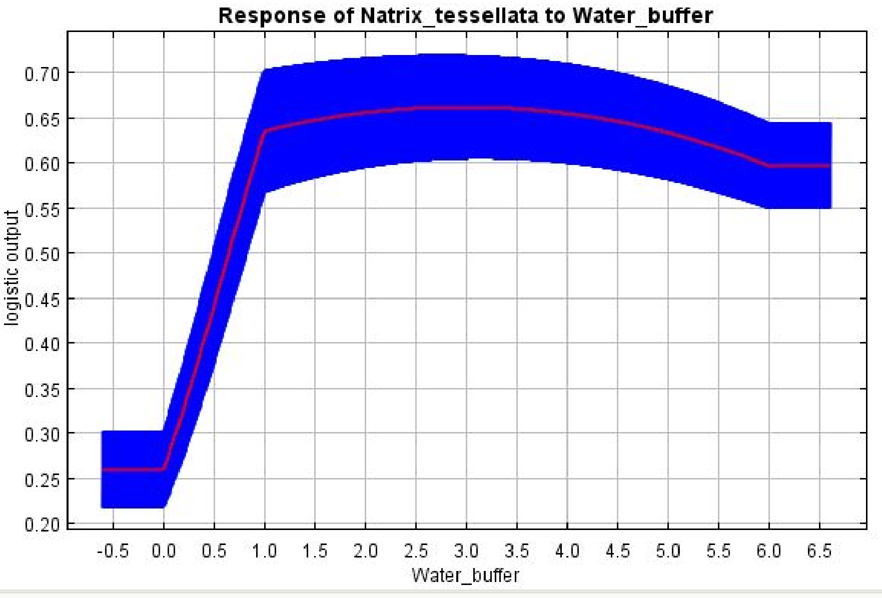

Figure 2. doi

Response curve of the watercourses and -bodies variable (including 200 m buffer around courses, y-axis: 1-3 watercourses ascending with course size, 4-6 waterbodies, ascending with body size).

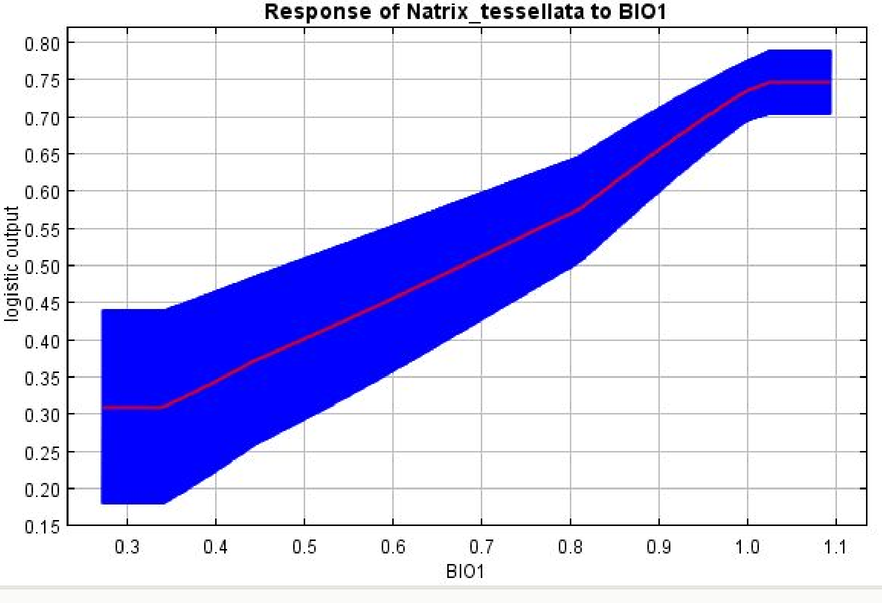

Figure 3. doi

Response curve of the annual average temperature (°C / 10).

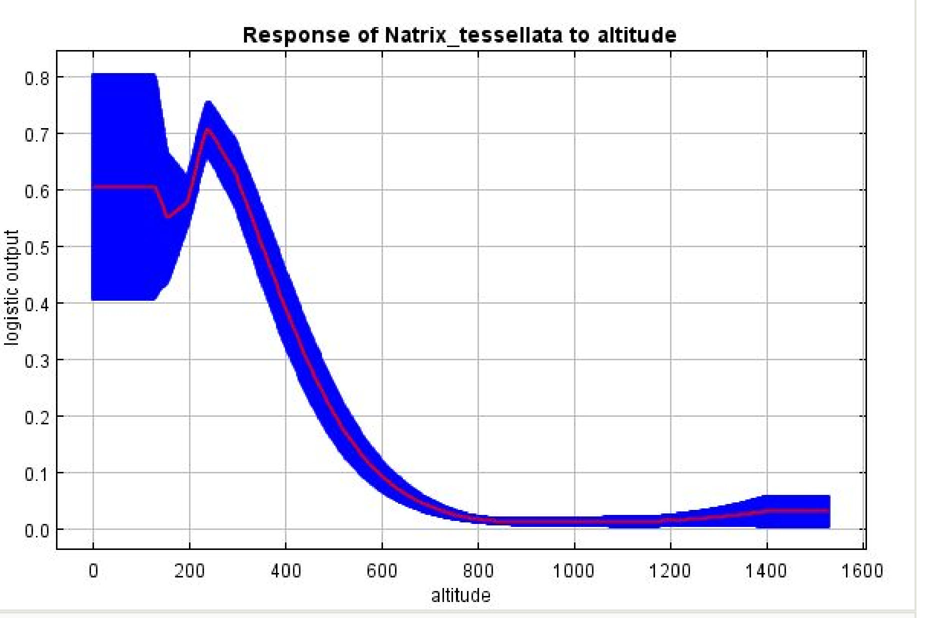

Figure 4. doi

Response curve of altitude (m a.s.l.).

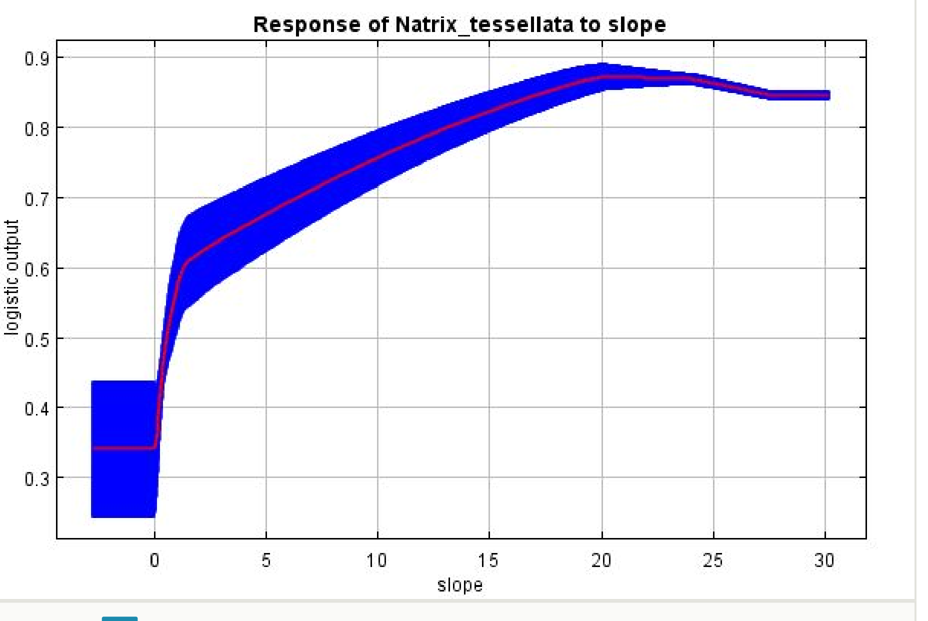

Figure 5. doi

Response curve of slope (°).

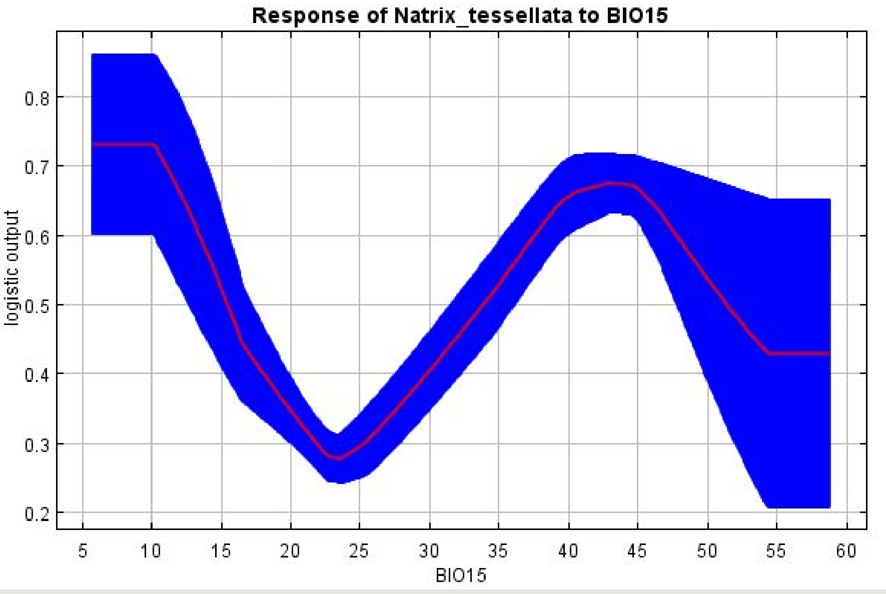

Figure 6. doi

Response curve of BIO 15 - Precipitation Seasonality (Seasonality Index).

According to the model, the probability of presence of *N. tessellata* is highest in areas up to 200 m from watercourses and -bodies (Fig. 2) in areas with annual temperatures of 10-11°C (Fig. 3). The probability of presence sharply drops in altitudes 250-400 m a.s.l. (Fig. 4) and is highest in areas with slope inclination of 20-25° (Fig. 5). The probability of presence is also highest in areas with the Precipitation Seasonaility index (Walsh and Lawler 1981) of 0-10 and 40-45 (Fig. 6).

Discussion

The predictive strength of the model was very high (mean AUC = 0.92). Only 8% of the actual real presence points were located below the predicted occurrence threshold, including several remote presence points from areas where *N. tessellata* occurrence is unlikely – for example, area near Česká Třebová and Zábřeh in the East Bohemia/West Moravia or Volary and Vimperk in the South-western Bohemia. These points are listed in the national database as taken over from sources labelled as controversial. The number of these points is negligible and we consider it a price for a large data sample from a wide spectrum of informants. Since the database is only open for authorised zoologists, included records are reliable and the size of the input dataset contributes to the prediction strength (Hernandez et al. 2006, Merow et al. 2013).

The probability of presence by annual average temperature is highest at 10-11°C. This result is in full accordance with the published data and with the fact that significant areas with higher temperatures are hotspots of the actual *N. tessellata* distribution (Mikátová et al. 2001, Moravec 2015). So far, there is no indication from the core area of distribution that higher temperatures are restraining the presence of the species. The average and maximum temperatures on the majority of the area are gradually increasing from 1961 (Zahradníček et al. 2020). The increase seems to be even faster in years 2011 to 2019 and these increases are most severe in the already warmest areas of the Czech Republic with the increase as high as 0.43°C per 10 years. This could mean significant future changes of suitable habitats for many species, including reptiles.

The predicted presence probability increased with proximity to watercourses and -bodies. This corresponds with publications about populations at or beyond the northern edge of the species continuous area of distribution (Mikátová et al. 2001, Moravec 2015) and, apart from species ecology, this can be explained by the fact that the Czech *N. tessellata* populations are linked to the so-called river phenomenon (Jeník and Slavíková 1964, Vannote et al. 1980, Ložek 1988, Ward 1998, Mikátová et al. 2001). The specific geology and temperature, water and air regime of deeply incised river valleys allow the occurrence of thermophilic organisms beyond the northern boundary of their continuous range on river slopes with southern exposition.

Increasing probability of prediction with decreasing altitude and increasing slope inclination supports the suspected link of the distribution to river valleys and its importance was closely followed by altitude. Near the northern edge of the species distribution range, *N. tessellata* inhabits mostly altitudes of 200-350 m a.s.l. (Mikátová et al. 2001, Moravec 2015). This corresponds with the model, where the probability of prediction sharply drops above approximately 400 m a.s.l., although *N. tessellata* can be found even higher in the mountains in the southern parts of its range (Piemonte, Italy up to 2000 m a.s.l., Austria up to 840 m a.s.l., Bulgaria up to 1420 m a.s.l., Asia up to 2800-3000 m a.s.l. (Rehák 1992, Nistri et al. 1997, Grillitsch and Cabela 2001, Stojanov et al. 2011).

Precipitation was expected to influence the species distribution, given that Czech *N. tessellata* populations are piscivorous and closely related to rivers and waterbodies as a source of prey. The highest probability of presence in areas with a Seasonality Index (Walsh and Lawler 1981) of 0-10 (precipitation spread throughout the year) and 40-45 (rather seasonal with a short drier season) corresponds to the avoidance of excessively humid locations for reproduction and wintering (Mikátová et al. 2001, Moravec 2015). There is also a strong assumption of intercorrelation of average annual precipitation with average annual temperatures (Schultz and Halpert 1993).

The link to the river phenomenon was well documented for *N. tessellata* distribution in Bohemia (Ložek 1988, Mikátová et al. 2001, Moravec 2015). The regional pattern of distribution suggests historical contraction of the range following the period of the Holocene climatic optimum with *N. tessellata* populations surviving in these refugia (Guicking and Joger 2011).

The resulting map of the species presence in Bohemia is in full compliance with the occurrence of the river phenomenon in the Czech Republic. In this model, the precipitation variable explains a large portion of variability, but only when combined with other significant variables. Examples of *N. tessellata* from localities in Bohemia (isolated from the continuous area of species distribution) and from localities in South Moravia (north-western border of the continuous species range) are shown in Figs 7, 8, 9, 10.

Figure 7. doi

Picture of a *N. tessellata* biotope in Bohemia (Nezabudické skály Natural Reserve).

Figure 8. doi

Natrix tessellata individual from a population in Bohemia (Nezabudické skály Natural Reserve).

Figure 9. doi

Picture of *N. tessellata* biotope in South Moravia (Podyjí National Park).

Figure 10. doi

Natrix tessellata individual from a population in South Moravia (Podyjí National Park).

The most recently published map of distribution (Moravec 2015, Šandera 2023) is in good consistency with our predictive model, but since it is a square network map without precise locations and coordinates, a more detailed comparison is problematic – especially with regard to the extraordinarily variable geomorphological relief of the Czech Republic and to the distribution of *N. tessellata* in the Czech Republic, where there is a strong correlation with the geomorphological relief variability – in this case, the network mapping easily includes the areas where the species is not present.

The link of Bohemian *N. tessellata* populations to specific microclimatic parameters and terrain morphology suggests that the most effective conservation strategy should be protecting their actual and predicted habitats. Additionally, the habitats linked to river phenomenon seem to be amongst the most resistant to climate change which otherwise poses a major threat to reptile populations (Sinervo et al. 2010, Dubey et al. 2012). Since climatic data from a large area have lower resolution, we recommend to analyse climatic parameters and their changes on a smaller scale, for example, several populations within areas with the highest temperature increases.

Conclusions

We consider the result of finding large areas with high probability of modelled prediction where real occurrence is not reported to be extraordinarily important. Here, we see the need to direct species monitoring to these places and search for historical data. These sites should be considered as a matter of priority for possible repatriation efforts and for conservation management by species introduction if the nature conservation authority decides for them. Additionally, these areas might be possible corridors for migration. In recent history, reptile species have been observed to occur beyond the northern range of their continuous areas (Jablonski et al. 2014, Rehák et al. 2022) with the possibility of both natural migration and human introduction. This expansion could also provide opportunity for hybridisation with related species (Asztalos et al. 2021, Schöneberg et al. 2023). The areas pinpointed from the model should also be subject to analysis of human footprint and possible dangers, for example, new cyclist corridors are being constructed along major rivers leading to heightened mortality and turning promising migration corridors into ecological traps.

The comparison of our predictive model and real distribution shows that the predicted and real distribution are almost fully accordant. Thus, a creation of predictive distribution models is a helpful instrument to facilitate monitoring and conservation efforts.

Conflicts of interest

The authors have declared that no competing interests exist.

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Supplementary materials

Suppl. material 1: Bioclimatic variable corellation matrix doi

Authors: Jan Chmelař, Veronika Rudolfová
Data type: statistic
Brief description: This file contains correlation matrices for the Pearson correlation coefficient (r), Pearson coefficient of determination (r^2) and Variance Inflation Factor (VIF) for bioclimatic variables used in the model.
Download file (38.60 kb)

Suppl. material 2: MaxEnt model output doi

Authors: Authors team
Data type: distribution
Brief description: Pdf. file showing cross-validated output of triplicate MaxEnt model.
Download file (3.68 MB)

Analysis of microhabitat preferences in the European green lizard (*Lacera viridis*) – a tool for conservation management of isolated populations

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Abstract

The European green lizard (*Lacerta viridis*) populations in Bohemia are located beyond the northern border of continuous range of this species, are stenotopic, closely linked to very specific habitats in deeply incised river valleys (the so-called river phenomenon) and isolated from each other. The chosen research site is the subject of long-term conservation management aimed at strengthening and maintaining abundance of the local population. In order to formulate a generalizable model for the management of other isolated *Lacerta viridis* populations, the authors performed a spatial analysis of the places with presence of an observed individual in order to determine and evaluate significance of the chosen biotic and abiotic factors for habitat discrimination. The results indicate that positive discrimination is based on the presence of a rock debris and a hiding place. Strongest factors towards negative discrimination were high percentages of grass and high vegetation coverage. The model is well applicable in both theory and practice of the species conservation and population management.

Key Words:

conservation modelling, spatial analysis, discriminant function analysis, population ecology, population characteristics, regional stenotopy, climate change

Introduction

Species distribution is often modelled and analyzed on a large scale (Kaliontzopoulou et al. 2008; Sillero et Carretero 2013; Oraie et al. 2014; Hosseinian Yousefkhani et al. 2015; Wirga et Majtyka 2015; Vargas-Ramírez et al. 2016; Petrosyan et al. 2020; Chmelař et al. 2020, 2023; Srinivasulu et al. 2021). Due to ecological specifics, reptiles may also show major differences in microhabitat preference and usage. Both these aspects need to be taken into account for an effective management of their habitats and populations.

Studies based on positive or negative discrimination of selected factors are common in botanical works, but have recently been applied to reptiles (Sacchi et al. 2011) for occurrence prediction. A similar analysis can be used to separate sympatrically occurring species according to their ecological demands (Melville et Swain 1997, Heltai et al. 2015), or to confirm the search for or avoidance of certain environmental factors, for example invasive plant species (Hacking et al. 2014).

In the Czech Republic, the European green lizard, *Lacerta viridis* (Laurenti, 1768), is generally rare and declining, as a result of habitat degradation, and its survival in this location is uncertain (Baruš et al. 1989, 1992; Mikátová et Nečas 1997; Mikátová 2002; Moravec 2015; Rehák 2015; Mikátová et Jeřábková 2023). All populations in the Czech Republic should belong to the nominotypical subspecies *Lacerta viridis viridis* (Böhme et al. 2007b). According to legislative regulations in the Czech Republic, the European green lizard remains listed among critically endangered species even though the current Czech Red List decreased the category to endangered (Chobot et Němec 2017). The reason for this change is a generally good state of populations in the Moravia region in the Southeast in contrast to populations in the Bohemia region. However, the biggest difference between Bohemian populations and populations from South Moravia is the noticeably lower genetic diversity, heterozygosity rate and allele richness of these populations (Böhme et Moravec 2011) as opposed to the populations in the core area (Nemitz-Kliemchen et al. 2020). This is apparently a consequence of geographic isolation of these population and an important aspect for their conservation and management.

Molecular data confirmed genetic affinities of Bohemian populations to those in neighboring parts of their continuous distribution range in NE Germany (Elbe River) and Moravia (Böhme et al. 2006; Böhme et Moravec 2011). No recent records of *L. viridis* presence is known from Poland (Skawiński et al. 2019). Moreover, individual relic Bohemian populations are also more or less isolated from each other and genetically slightly distinct (Böhme et al. 2006; Böhme et al. 2007a; Böhme et al. 2007b; Böhme et Moravec 2011). These populations are ecologically notable as inhabitants of biotopes retaining ancient characteristics and they can differ significantly in ecological parameters (Strödicke 1995, Joger et al. 2010; Fischer et Rehák 2010; Blažek 2013). These habitats are mostly highly fragmented (Prieto-Ramirez et al. 2018).

All Bohemian populations are bound to the so-called "river phenomenon" (Ložek 1988). Populations are geographically isolated from each other, even from a continuous southern distribution. The idea of the formation of metapopulations is therefore not very likely. This creates the possibility of comparing these individual populations, both in terms of morphology, ecology and ethology. These are also populations showing regional stenotopy, linked to specific biotopes at the northern limit of species distribution and occur sympatrically with other animal and plant species that are connected with the river phenomenon (Ward 1998; Chmelař et al. 2023). The data on the ecology obtained from these localities are therefore very valuable, as the

European green lizards are probably found here at the very ecological limits of the species. The Bohemian relict autochthonous populations of *Lacerta viridis* have extraordinary scientific and conservation value, and due to their genetic exceptionality related to isolation, fragmentation, small population size, genetic drift, reduced variability and the possibility of occurrence of unique genetic variants, they also require special methods of conservation management (cf. Böhme et al. 2007a, Joger et al. 2010).

The distribution of *L. viridis* in the Czech Republic has already been analysed and a prediction model has been developed to identify suitable habitats on a large scale (Chmelař et al. 2020). The aim of this paper is to identify the key factors influencing microhabitat selection, to evaluate if these factors correspond on both scales, and to contribute to the practice and theory of conservation of isolated populations in general.

Material and methods

The research was carried out in the valley of the Únětický stream, otherwise also called Tiché údolí (the Silent Valley). The entire location is located on the border of Prague and the Central Bohemian region. The valley has the local character of a gorge. The whole sampling area is part of the Roztocký háj –Tiché údolí Nature Reserve and is protected by national laws.

From a geological point of view, the monitored area falls into the area of the Barrandien Paleozoic, sedimentary rocks, especially shale and silicite, predominate. The filling of the valley consists of alluvium deposits on sandy gravels (Fediuk 1997).

The location of the European green lizard is a south-facing slope with an area of 4.2 ha consisting of 2 abandoned quarries and the slope itself. Its important parts are fragments of heaths and rocky steppes with native flora on rocky outcrops. Such diverse terrain includes a considerable number of microclimates with relatively high temperature differences. There are frost basins in the area of the valley floor, with a frequent temperature inversion, especially in spring. In contrast, the exposed rock outcrops show significantly higher temperatures than would be usual for the given time of year.

For easier orientation and differentiation of individual sub-biotopes, the monitored area was divided into smaller parts. A linear transect with a total length of 1.9 km was laid out through these parts, and all the individuals detected along this transect were recorded. The obtained data were also used for mapping the annual and daily activity, for estimating the number of the population and as an indicator of the relative composition of the population in terms of gender ratios and age categories. In total, 403 presences were recorded in the years 2011-2014 with the total of 119 visits to the site.

GPS coordinates of the points of presence was measured using a Trimble GeoXT GPS receiver. The recorded measurements were further enhanced by geodetical software. The estimated accuracy of the measured points is within 50 cm in 87.6% of cases, within 1 m in 9% and only 3.4% of the measurements have an expected deviation greater than 1 m.

These points of recorded presences were used to perform the spatial analysis. The mapping was carried out during June and July 2014 in order to minimize seasonal differences in microhabitat layout. Also, the variables were selected with minimizing the effect of seasonal change in mind. Especially in the case of variables related to vegetation, we focused on the percentage of their coverage and/or number, rather than to the exact height, or the degree of shading of the surface. Two types of variables were mapped in the field, namely:

1. Percentage representation of the given surface within a radius of 0.5 and 2.5 m from the point of recorded presence. The following factors were mapped: scree, grass, soil, tall vegetation, raised rock, stump or fallen log, leaves and branches.

2. The number of given objects within a radius of 0.5 and 2.5 m from the point of occurrence. The following objects were mapped in this way: bush, thornbush, tree, raised rock, stump or fallen log, shelter and deep shelter.

Factors with unclear definition were arbitrarily standardized according to their assumed ecological function, not according to systematics. Tall vegetation includes herbs higher than 30 cm. A raised stone is defined as having a minimum length of 30 cm in its longest dimension and an elevation of at least 15 cm compared to the surrounding surface, rock outcrops were also classified in this category. A shrub was scored as any woody plant up to 2 m tall that was sprouting close to the surface and could therefore provide shelter from a potential predator. As trees were classified woody plants from a height of 2 with branches high above the ground and did not provide direct shelter near the surface. Shelter was defined as any subsurface space in which an individual is able to hide. In the case of a deep shelter, it was assumed that it could also serve as a place for wintering or a laying a clutch of eggs.

For comparison with the above-mentioned presence records, we created a total of 200 random points within the same area using the QGIS software. Selected variables were recorded in the vicinity of these points using the same method as above.

Prior to the analysis, variables were screened for spatial intercorrelation using the Mantel test. Principal component analysis (PCA) was also performed to identify redundant variables. Both types of points were then compared using discriminant function analysis (DFA) in the STATISTICA software, using the presence as the grouping variable (value of 0 for random point and 1 for a point of recorded presence. The final model was constructed by a method of backwards stepwise variable elimination. Another DFA was afterwards performed with the age category (adult, subadult, juvenile) as a grouping variable in order to identify possible differences in their microhabitat structure. Again, backwards stepwise variable elimination was used.

In the case of a comparison without considering the category of the individual, the probability of correct classification would be 50% for a random sample (random point and a presence record). The probability of random classification was specified in the statistical method to be the same for all categories, i.e. in the case of random distribution 33.3% for each of the 3 age categories.

Results

The Mantel test identified variables with r > 0.2 Only the percentage of soil in the radius of 0.5 and 2.5 m from the presence record had exceeding values of intercorrelation and were not included in further analyses.

1. DFA of presence records and random points

Unreduced model shows significant differences between random and recorded presence points (Wilks' Lambda: 0.577, $F_{(24.578)} = 17.69$ $p < 0.0001$).

Then we reduced the number of variables in the model by backwards stepwise elimination method and 9 variables have remained in the model: scree percentage 0.5 m, grass percentage 0,5 m, high vegetation 0.5 m, branches percentage 0.5, elevated rock/stump percentage 0.5 m, number of trees 0.5 m, branches percentage 2.5 m, number of shelters 2.5 m, number of deep shelters 2.5 m.

Wilks' Lambda of the reduced model (0.598) remained significant ($F_{(9,593)} = 44.24$ $p < 0.0001$). The success of the classification is summarized in Tab. 1. The model was able to classify the random points correctly in 82.5 percent of cases (165 out of 200). The classification success rate of points of presence was 79,9 % (322 out of 403).

Tab. 1: Classification Matrix of DFA analysis, Rows: Observed classifications Columns: Predicted classifications

	Percent	Random	Presence
Random	82,5	165	35
Presence	79,9	81	322
Total	80,8	246	357

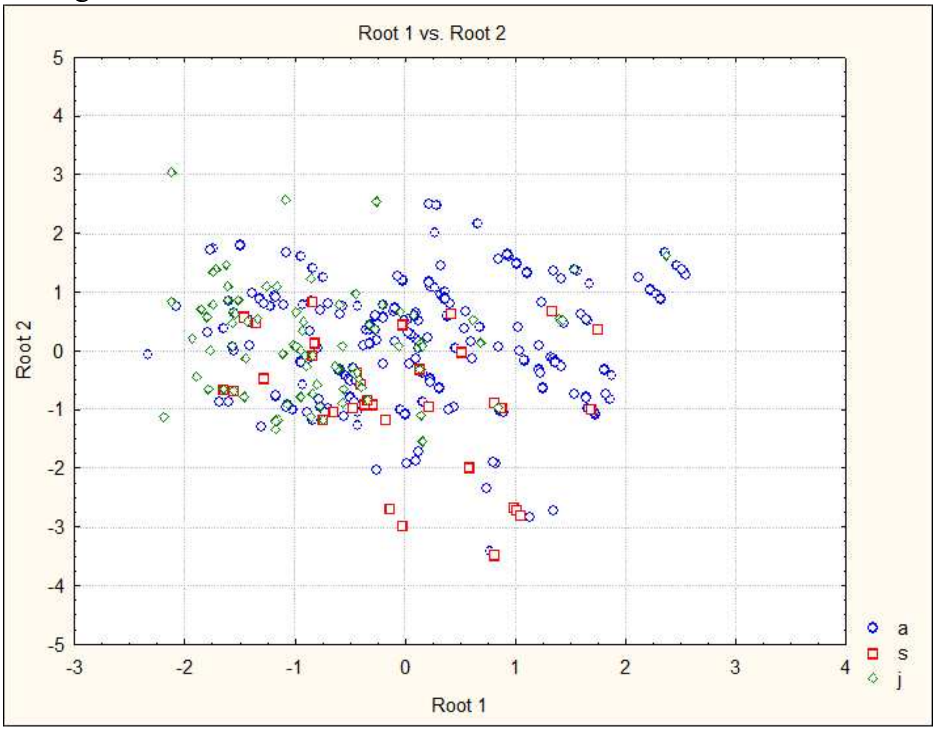
2. DFA of age categories the unreduced model (Wilks' Lambda: 0.75, $F_{(48,754)} = 2.392618$, $p < 0.00001$) shows significant differences in classification between points of presences of adults and juveniles:

(Squared Mahalanobis distance = 1.25, F = 3.52, $p < 0.00001$) and between points of presences of juveniles and subadults (Squared Mahalanobis distance = 1.60, F = 1,65, p = 0.03). The classification success rate is shown in Tab. 2 and cluster visualization is shown in Fig. 1.

Tab. 2: Classification Matrix of DFA analysis with age category as grouping variable, Rows: Observed classifications, Columns: Predicted classifications

	Percent	a	s	j
a	88,4	236	4	27
s	13,5	27	5	5
j	35,4	64	0	35
Total	68,5	327	9	67

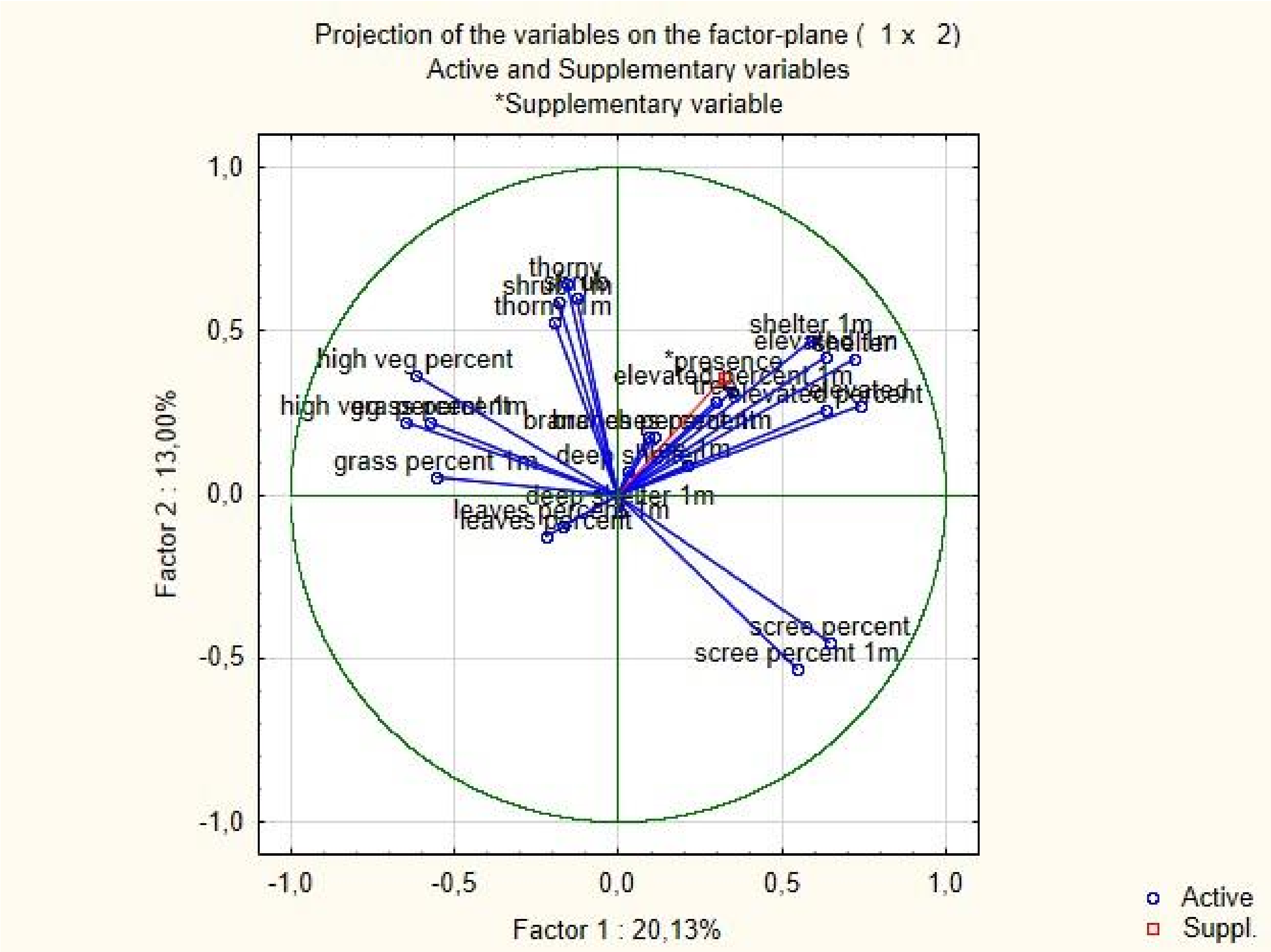
Fig. 1: Visualisation of canonical analysis according to age categories. The result shows no distinguishable clusters.



3. Principal Component Analysis (PCA) of all variables

Through principal component analysis, from the PCA scree plot, 5 factors explained significant percentage of variability. The first 2 factors were selected as determinants, which are graphically represented in the form of axes in Figures 2 and 3. The first factor explained a total of 20.13% of the internal variability, while the second factor explained 13%. Four main vectors were identified by projecting the variables onto the plane.

Fig. 2: Projection of PCA into factor plane



Discussion

Regardless of the chosen statistical method, all the analyses results show that the distribution of individuals in the monitored locality is not random.

The output of discriminant analysis shows that the created model is reliably able to distinguish a point of presence from a randomly selected point. However, it can´t reliably classify the presence points of individuals into the correct age category. There was a significant discrimination between juveniles and adults and between juveniles and subadults, but the classification success rate was relatively small. Nevertheless, the change of habitat preference by *L. viridis* during ontogeny is widely recognized and supported by the published data from many populations (e.g. Fischer et Rehák 2010; Harta et al. 2017).

The sex of adult individuals was not evaluated in the analysis due to no significant difference in the success of classification of presence points of males and females in preliminary analyses.

After discarding redundant variables, nine statistically significant key environmental factors remained. In the 50 cm radius of the presence of the animal it is the scree percentage, grass percentage, high vegetation, branches percentage, raised rock/stump percentage, number of trees, proportion of debris, grasses and tall vegetation (not shrubs, which were evaluated as a separate variable). The proportion of high vegetation and grasses was higher at random points, while a higher representation of debris, on the other hand, was more characteristic of points with the presence of lizards. At a distance of up to 2.5 m, following variables remained after the reduction, branches percentage, number of shelters, and number of deep shelters which characterized the points of real presence.

According to the results of the analysis, it is precisely these variables that are able to distinguish multidimensional groups of objects, in this case points on the monitored location. These results should be interpreted with great caution. The European green lizard is a relatively large and very mobile species. A circle within 50 cm directly from the place of presence would therefore show which surface is directly chosen by the animal for its current position. Factors at this distance will therefore be important mainly in terms of thermoregulation and passive antipredation. From the point of view of prey accessibility, but especially active antipredation, the vicinity of a point at a greater distance is much more important. The value of 2.5 m was not chosen randomly, it is the approximate maximum distance that an adult individual was able to run in one partial run without stopping. In this environment, the authors assumed discrimination according to the presence of a potential long-term shelter.

From the PCA visualization (Fig. 2), four vectors can be recognized.

1. The first vector which corresponds with the direction of recorded presence, contains mainly variables of elevated rock and shelter (both within 0.5 m and 2.5 m from the point of recorded presence) this vector seems to include variables directly linked to antipredatory/thermoregulatory function. Elevated position provides a basking opportunity and vantage point to see potential predators or competitors while the availability of shelter in the immediate vicinity is necessary to avoid predation (Majláth et Majláthová 2009, Fischer et Rehák 2010).
2. The second vector consists of bush and thorny bush variables and we interpret these variables as mainly antipredatory. The vector is directed roughly in accordance with the recorded presence, which corresponds with data from similar studies (Heltai et

al. 2015). Lizards have been frequently observed running first into a nearby shrub if disturbed and only if pursued further, seeking a refuge in subterranean shelter. This interpretation can also be supported by a fact that 95% of recorded presence points had at least one shrub or shelter within 50 cm and 99% had at least one shrub/shelter within 2.5 m. We found no difference between usage of thorny/not thorny shrubs.

3. The third identified vector contains grass and high vegetation variables and does not correspond with the direction of recorded presence. Preferred explanation is that high vegetation and grass provide shade and thus lower the temperature of surfaces covered by them. Also, these objects limit the lizard field of view without providing a substantial cover form the predators, posing a potential risk. Of course, during periods of supraoptimal temperatures, individuals have been observed seeking lower temperatures in shade, but mostly preferring a shade provided by shrubs or seeking a subterranean shelter.
4. The last identified vector consists only of the scree coverage (both within 0.5 and 2.5 m) and does not correspond to the presence, but neither goes in the opposite direction. This is interpreted as mainly thermoregulatory effect since the scree can be very easily warmed by the sun, but these surface temperatures can in warmer periods on direct sunlight easily exceed 80° C which makes them unusable. The gaps between scree are sufficient for juveniles and most subadults to be used as cover from predators, but larger adults are not able to fit in most of them. The rising percentage of warmer days in the area (Zahradníček et al. 2020) could also lead to switch in both microhabitat and habitat usage in the future (Rehák et al. 2022) Thus, we consider scree to be preferred surface only under very specific circumstances.

The nature of the site must also be considered when interpreting the results. The random points were plotted into the polygon covering the site (4.2 ha) and did not include any areas inaccessible to lizards. There was therefore no risk that a random point would be placed, for example, in the middle of a stream or beyond the boundary of the site. Due to the relatively small size of the polygon, it is therefore necessary to assume that a significant number of the random points was located in the immediate vicinity of the points of recorded presence, which was also often the case. Despite this assumption, the analysis was able to distinguish the vast majority of random points from real observation locations.

For the purpose of the analysis, individuals have not been individually recognized, this means that the dataset could contain some pseudoreplications by recording the same individual multiple times. This problem could not be avoided, but was minimized by using the line transect method, thus significantly lowering probability of repeated records. There was also a time difference between individual site visits, lowering the risk even more.

Multivariate statistical models are rarely used in the study of reptile habitats. This method is widespread especially in botany, specifically it is often used to predict the occurrence of selected species. However, in studies focused on aspects of species protection, these are very valuable methods, the outputs of which can have direct application. For example, discrimination of a certain type of habitat in reptiles (Hacking et al. 2014) using the MANOVA method. The output of their analysis was that Schmeltz's skink (*Carlia schmeltzii*) avoids microhabitats with a high proportion of invasive grasses.

A similar method was also used in the study of the local population of *L. bilineata* in northern Italy (Sacchi et al. 2011). According to the authors, individuals in the monitored population purposefully seek out ecotones for their microhabitat, however, they do not discriminate based on the specific composition of these ecotones. Other authors also mention the importance of ecotones in *L. viridis* microhabitat usage (Harta et al. 2017).

In the previous study, we identified 4 factors that showed positive influence on the species distribution in the Czech Republic: annual precipitation up to 600 mm, slope inclination between 5-25°, mean temperature of the warmest quarter up to 20 °C and precipitation in the coldest quarter above 150 mm (Chmelař et al. 2020). These factors seem to well describe the preferred habitats that can support the *L. viridis* populations including our chosen research site.

Photos of a habitat and an individual from the research site are shown in Fig. 3 and Fig. 4. The research site is a subject to an active management since 2000 and in 2013, both the population density and area usage significantly increased in comparison to data from years 1995-1997 (Fischer et Rehák 2010) with the density and abundance corresponding to populations in similar habitats (Prieto-Ramírez 2023), still the isolation of the population means a high risk to its long-term survivability (Böhme et al. 2007b) and small isolated populations are presumed to be most threatened by habitat erosion due to the climate change (Sinervo et al. 2010). The management measures were focused on keeping the landscape mosaic with retaining of key microhabitat elements while avoiding excessive growth of vegetation coverage, ideally by combining grazing and cutout (Fischer et Rehák 2010; Rehák 2015; Fischer et al. 2016, 2023; Mizsei et al. 2023). Our study has identified some of these microhabitat elements and their combinations which should be taken into account when planning management measures in similar areas.

Fig. 3: Typical habitat of *Lacerta viridis* (maintained by active management). Roztocký háj - Tiché údolí Nature Reserve.

Fig. 4: Adult male *Lacerta viridis* from the Roztocký háj - Tiché údolí Nature Reserve

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Can predictive modelling of species distribution explain population decline? Study on the sand lizard, *Lacerta agilis*, in the Czech Republic.

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Abstract.

The sand lizard, *Lacerta agilis* has been, until recently, considered a common and abundant species in the Czech Republic. However, a significant decline has been documented in recent years and several hypotheses have been raised to explain it. We created a predictive model and determined key factors influencing the species distribution in the Czech Republic. The most relevant factors were: mean annual temperatures, altitude and proximity to water courses or bodies. The model is in full accordance with the presence records and can be applied in both theory and practice of the species conservation, e.g. identifying areas with preferred habitat to focus species monitoring and management efforts. The model also suggests the vulnerability of the identified habitats to climate change and interspecific competition with other reptile species. It is finer than coarse grid maps and is less affected by differences in monitoring effort than spot maps, shows that *Lacerta agilis* can occur in the vast majority of the territory of the Czech Republic as a highly euryecious species, and thus demonstrates that the causes of its decline here are not significantly linked to geographical or climatic conditions, and indicates that the causes of the decline of *Lacerta agilis* must be sought elsewhere. As an ecologically pioneer species, it can be displaced by successive habitat changes. In areas of sympatric occurrence, *Lacerta agilis* may be displaced by the locally more competitive *Lacerta viridis*. In general, we consider the most serious cause of the decline of *Lacerta agilis* in the Czech Republic to be the homogenization of the landscape and the dramatic decline of suitable habitats occurred as a result of the creation of large agricultural and urbanized areas.

Key words. Squamata, Lacertidae, species conservation, habitat management, inter-species competition, climatic change.

Introduction

Studies using predictive modelling of species distribution are a valuable tool in understanding specific climatic and geographical factors that affecting it (Elith et al. 2006, Civiš 2013). These analyses are nowadays common in analyzing the distribution of reptile and amphibian species (e.g. Kaliontzopoulou et al. 2008, Sillero & Carretero 2013, Oraie et al. 2014, Hosseinian Yousefkhani et al. 2015, Wirga & Majtyka 2015, Petrosyan et al. 2020, Chmelař et al. 2020, Srinivasulu et al. 2021, Chmelař et al. 2023) and the impact of climate change on species distribution (Dubey et al. 2013), with a lot of reptile populations being small and isolated, thus vulnerable to habitat degradation due to a changing climate (Sinervo et al. 2010). These models can also help predict spreading of invasive species (Pyron et al. 2008, Jarnevich et al. 2018) and facilitate adequate measures by the competent authorities. In the presented case study on *Lacerta agilis* Linnaeus, 1758 in the Czech Republic, we also use the distribution model as an auxiliary tool to understand the causes of population decline.

The distribution of *L. agilis* in the Czech Republic is entirely within the range of the species (Bischoff 1984, Aghasyan et al. 2021). It can be found dispersed in suitable habitats all over the Czech Republic excluding high mountains, intensively used agricultural terrain and dense forests even though it can be found along the edges of these (Mikátová 2001, Zavadil et al. 2015, Jeřábková et al. 2017).

Compared to other Czech reptile species, *L. agilis* has a high ecological adaptability and can inhabit a wide range of habitats, even in suboptimal climatic conditions. It is considered a pioneer species that can quickly expand into newly deforested areas or areas with reduced vegetation coverage (Mikátová 2001, Moravec 2015, Mikátová & Jeřábková 2023). Until recently, the species was considered abundant in most parts of the Czech Republic even though some mentions of population decline are dated up to 1990s (Opatrný 1992). In recent years, Czech populations of *L. agilis* are rapidly declining in abundance despite the species presence being recorded from most of the areas of statewide quadrate grid mapping (MORAVEC 2015, MIKÁTOVÁ & JEŘÁBKOVÁ 2023).

Legislative regulations in the Czech Republic list *L. agilis* as Highly Endangered and it is Vulnerable according to the current Red List of Amphibians and Reptiles for the Czech Republic (JEŘÁBKOVÁ ET AL. 2017).

The Nature Conservation Agency of the Czech Republic is monitoring occurrence of reptile species since 2007 in order to map and understand their distribution. Published data of *L. agilis* presence are available from most parts of the state (MIKÁTOVÁ 2001, MORAVEC 2015, MIKÁTOVÁ & JEŘÁBKOVÁ 2023).

The aim of recent paper is to analyse available distribution records, describe the key factors influencing the distribution of *L agilis* and how these factors are affected by the climatic change in order to understand the recent population decline, and to create a predictive model that visualizes areas with suitable habitat for monitoring and management focus and identifies areas for potential species dispersal.

Material and methods

We used a state-wide database maintained by the Nature Conservation Agency of the Czech Republic as a source of *L. agilis* presence sites. The database contained 7737 records from the whole territory of the Czech Republic collected within the period from 1940 to 2014.

We created 73 layers in total for the modelling purposes covering the area of the Czech Republic: the lowest, highest and average temperatures for individual months (36 layers in total), precipitation in individual months (12), bioclimatic variables according to worldclim.org methodology (Tab. 1) (19), altitude, surface exposure, human footprint, slope, road network, water courses and bodies including a 200 m buffer (CIVIŠ 2013).

Tab. 1. Bioclimatic variables according to worldclim.org methodology.

Variable	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

WorldClim Worldwide database, which is routinely being used as the source of climate variables, only uses data from two meteorological stations for the whole Czech Republic (HIJMANS et al. 2005), this made it unsuitable for our research and we have created layers for the bioclimatic variables manually in ArcGis 9.3 (ESRI ArcGIS 2008) using the 2D coordinate system SJTSK Krovak East-North. The layers of climate variables were created, based on the Climate Atlas of Czechia (Czech Hydrometeorological Institute 2007), which includes data from 1961 to 2000.

Variables containing maximum, minimum and average temperatures and precipitation in individual months were not included in the model because of their high intercorrelation. In similar cases, a careful interpretation is recommended for making possible implications for species conservation (SYFERT et al. 2013). These data were also already included in the WorldClim bioclimatic variables.

The climatic variables were screened for intercorrelation in ENM Tools (WARREN et al. 2010), resulting in correlation matrices for the Pearson correlation coefficient "r", Pearson coefficient of determination "r " and Variance Inflation Factor "VIF" (Suppl. material 1).

Variables with r > 0.8, r > 0.8 and VIF > 10 were considered heavily intercorrelated (Pradhan 2016) and removed from the model not to be used alongside variables with which they were closely correlated. The final model included the following variables: altitude,
aspect, human footprint, road network, BIO 1, BIO 2, BIO 3, BIO 4, BIO 6, BIO 7, BIO 8, BIO 9, BIO 12, BIO 15, slope, water bodies and courses.

The Predictive Distribution Model was created using MaxEnt software (PHILLIPS et al. 2023), the output being a GIS document in .asc format. This software was specifically chosen to work well with presence-only data (ELITH et al. 2006, HERNANDEZ et al. 2006). As the *L. agilis* a presence prediction value, the “Logistic threshold” was used to optimize between the sensitivity of the model and the location of all real presence records in the predicted areas. MaxEnt model was run in three replications and automatically cross-validated. All other settings in the model were set to default.

Results

The predictive strength of the model (AUC) was 0.624 (62.4 % of actual presence records were above the prediction threshold). The mean “logistic threshold”, when the sensitivity of the model is equal to its specificity, was 0.482 higher value means that the model predicts *L. agilis* presence (see Fig.1). The most important contributors to the resulting model were: BIO 1 – Average annual temperature (explaining 22.1 % variability), altitude (21.5 %) and Buf200 - water courses with 200m buffer (12.6 %). Response curves of these variables are shown in Figures 2 to 4.

According to the model, the probability of presence of *L. agilis* is highest in areas with average annual temperatures of 10-11° C (Fig. 2), in altitudes up to 400 m a.s.l. and dropping up to altitudes of 1400 m a.s.l. (Fig. 3) and in areas up to 200 m from water courses (Fig. 4).

Fig. 1. Predicted distribution of the sand lizard (*Lacerta agilis*) in the Czech Republic.

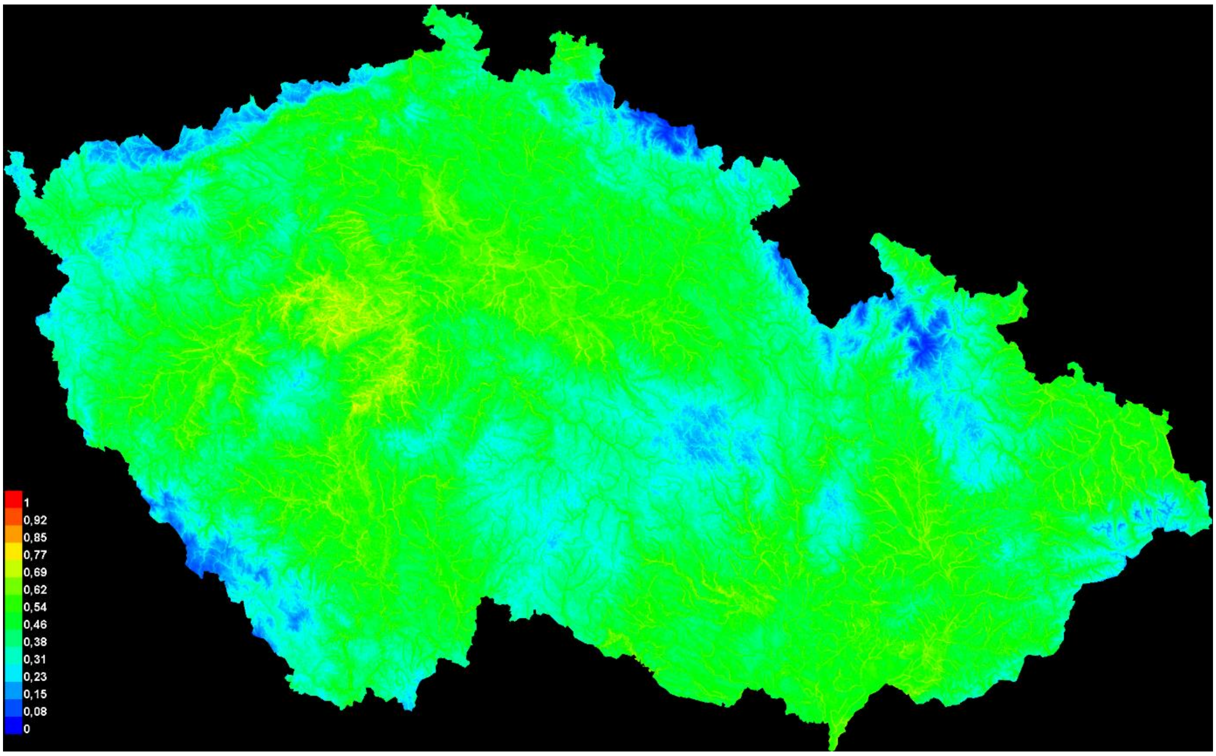

Fig. 2. Response curve of the annual average temperature (°C / 10).

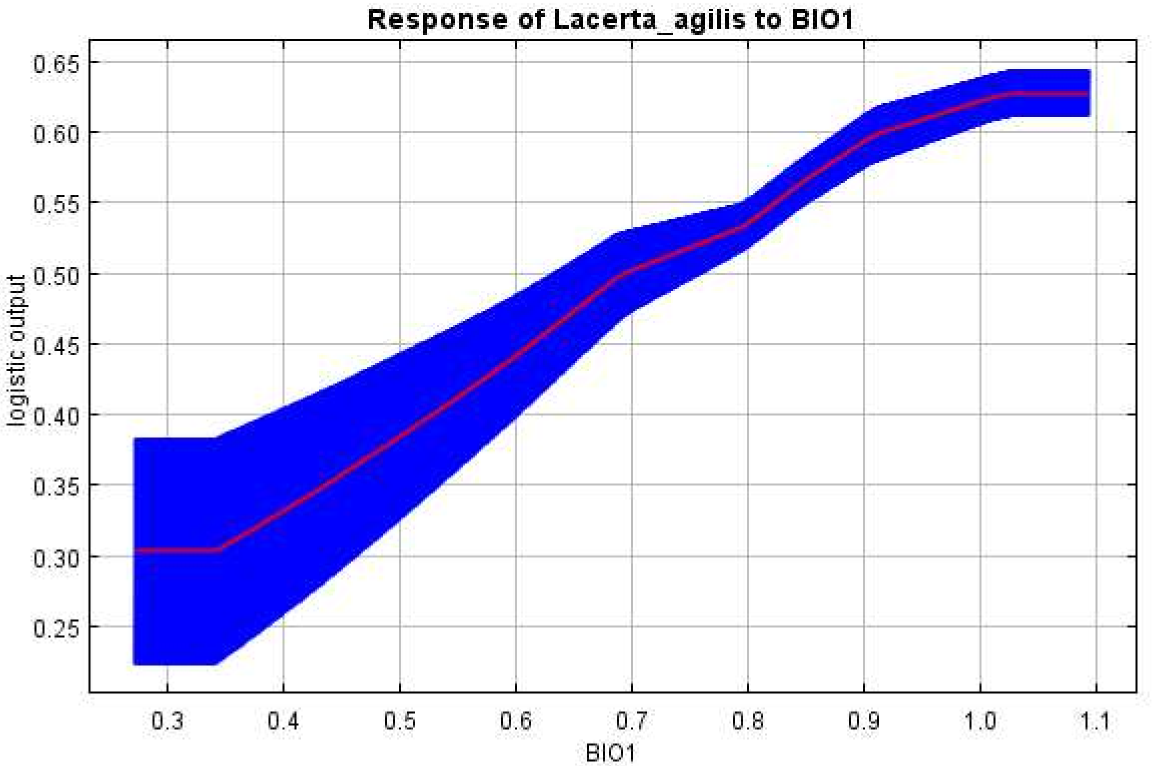

Fig. 3. Response curve of altitude (m a.s.l.).

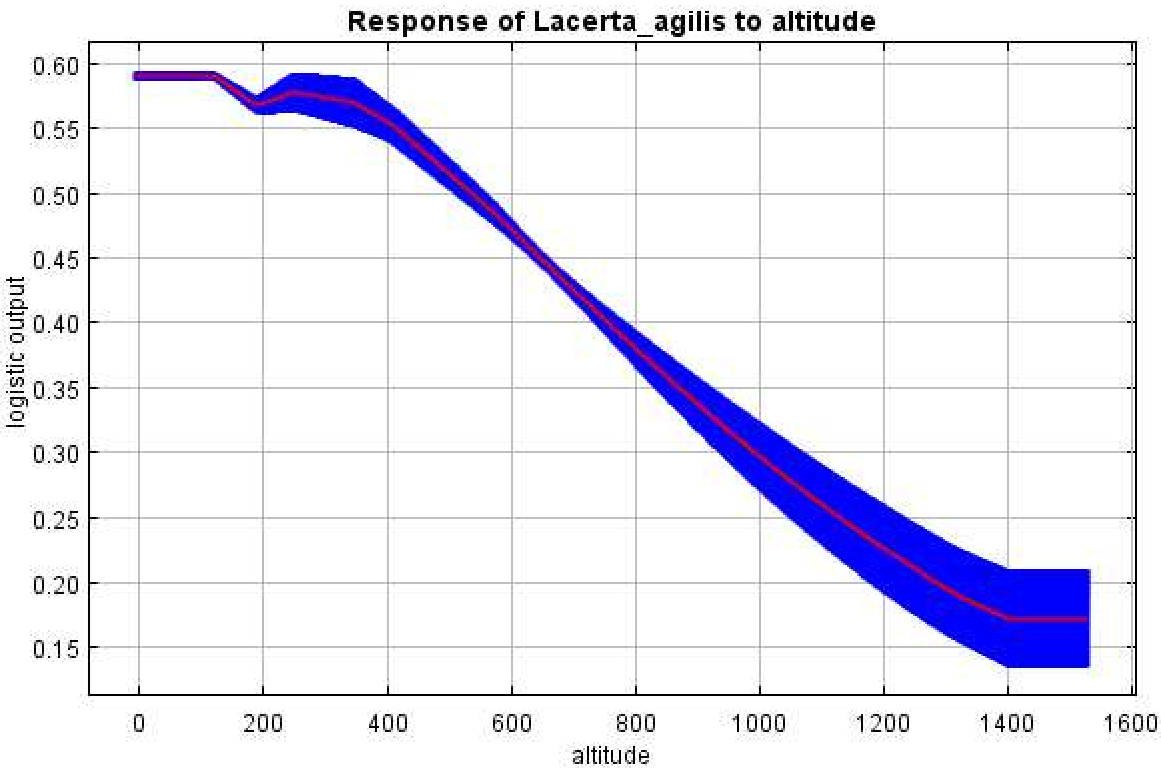

Fig. 4. Response curve of the water courses and bodies variable (including 200 m buffer around courses, y-axis: 1-3 watercourses ascending with course size, 4-6 waterbodies, ascending with body size).

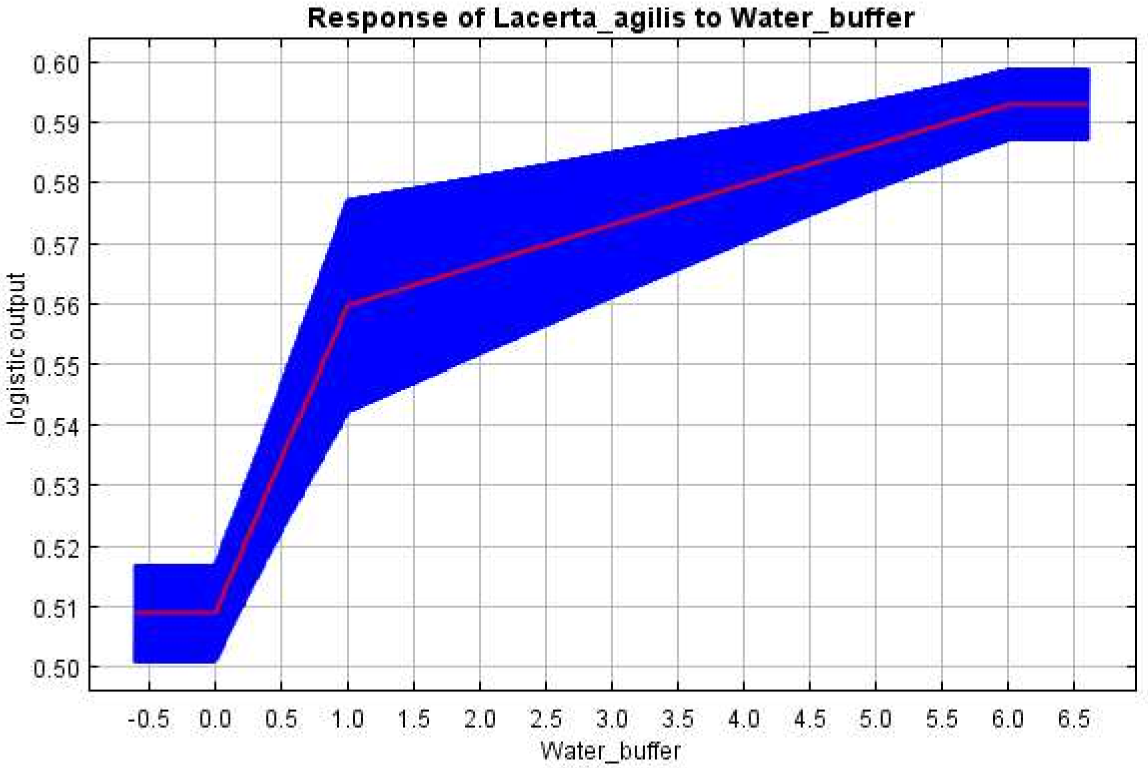

Fig. 5. Natural monument Neretský lom - the site of an abundant population of *Lacerta agilis*.

Fig. 6. Pair of *Lacerta agilis*, Neretský lom Nature Monument.

Discussion and conclusions

The predictive strength of the model was low (AUC = 0.624). Nearly 38% of the actual presence records were located below the predicted occurrence threshold, which further proves the width of the species ecological valence. These records included several areas where *L. agilis* occurrence is unlikely, even though it is recorded (at least historically) from 86 % of quadrates covering the Czech Republic (MIKÁTOVÁ 2001, MORAVEC 2015). The number of these points is negligible and we consider it a price for a large data sample from a wide spectrum of informants. Since inputs to the database is only open to biologists authorized by the Nature Conservation Agency of the Czech Republic, included records are reliable and the size of the input data set contributes to prediction reliability (HERNANDEZ et al. 2006, MEROW et al. 2013). The low prediction strength of the model can be explained by *L. agilis* being a habitat generalist, especially compared to other Czech reptile species. Distribution models for the European green lizard, *Lacerta viridis* (LAURENTI, 1768) and the dice snake, *Natrix tessellata* (LAURENTI, 1768) had very strong AUC values due to a close link to very specific habitats in the Czech Republic (CHMELAŘ et al. 2020, 2023). In accordance with this hypothesis, a more widely spread species would be much less likely limited by habitat constraints.

The probability of presence by annual average temperature gradually rises up to temperatures of 10-11° C which covers warmer areas in the Czech Republic. The species can partly compensate excessively warm periods by switching the diurnal activity to two daily peaks (morning and evening) (OPATRNÝ 1992, MIKÁTOVÁ 2001, BLANKE 2004, MORAVEC 2015). In recent years, there seems to be a trend of moving to colder areas (MIKÁTOVÁ & JEŘÁBKOVÁ 2023). The average and maximum temperatures on the majority of the area are gradually increasing from 1961 (ZAHRADNÍČEK et al. 2020). The increase seems to be even faster in years 2011 to 2019 and these increases are most severe in the already warmest areas of the Czech Republic with the increase as high as 0.43°C per 10 years. This could mean significant future changes of suitable habitats for many species, including *Lacerta agilis*. Thus, the effect of altitude on the species distribution was an expected result. This corresponds with the model, where the probability of occurence lowers with rising altitude and sharply drops above approximately 1400 m a.s.l. The highest recorded presence in the Czech Republic is from 940 m a.s.l. (Moravec 2015), but in western and central Europe, *L. agilis* can be found up to altitudes of 1700 m a.s.l. (GRILLITSCH & CABELA 2001, BLANKE 2004). As with the temperatures, in recent years, *L. agilis* seems to be gradually spreading to higher altitudes (MIKÁTOVÁ &

JEŘÁBKOVÁ 2023). The habitat shift has been previously described from the edges of the species range, but not from its core areas (SCHMITZ et al. 2022).

The predicted presence probability increased with proximity to water courses and bodies. According to the graph scale in Fig. 4, the increase in probability is low (From 50 % to 60 %), but most predicted hotspots of species presence are in the vicinity of rivers (Fig. 1). Although there is no specific mention of *L. agilis* being linked to rivers or water bodies neither by their trophic nor thermal biology, the preferred types of habitats (unforested with solitary shrubs and covered by low vegetation (MIKÁTOVÁ 2001, MORAVEC 2015, MIKÁTOVÁ & JEŘÁBKOVÁ 2023) are often found near water courses or bodies. The river network in the Czech Republic is very dense, so the buffer 200 m around water course and bodies covers a large area

The most recently published maps of distribution of *L. agilis* in the Czech Republic (MORAVEC 2015, MIKÁTOVÁ & JEŘÁBKOVÁ 2023, ŠANDERA 2024) are in good consistency with our predictive model, but these are square network maps where dimensions of one square are is 10 x 10 km which makes a detailed comparison problematic since the squares of this size often include areas where the species is not present. This method is also not suitable to study population ecology since even one presence record is enough to cover the whole square. The latest atlas of reptile distribution (MIKÁTOVÁ & JEŘÁBKOVÁ 2023) also includes a map of localities of species occurrence which is more comparable with the model. The advantage of the model is that it is finer than coarse grid maps and is less affected by differences in monitoring effort than spot maps. According to our model *Lacerta agilis* can occur in the vast majority of the territory of the Czech Republic as a highly euryecious species. At the same time, the model demonstrates that the causes of its decline here are not significantly linked to geographical or climatic conditions, and indicates that the causes of the decline of *Lacerta agilis* must be sought elsewhere.

In some areas, the occurrence of *Lacerta agilis* may be limited by competition with the European green lizard, *Lacerta viridis*. The present model of the distribution of *Lacerta agilis* shows that in the Czech Republic conditions suitable for this eurytopic species are also suitable for stenotopic *Lacerta viridis* (CHMELAŘ et al. 2020). However, the syntopic coexistence of both species (reported e.g. from Hungary - HELTAI et al. 2015) is exceptional here, and in the case of sympatric occurrence segregation is evident by the selection of different habitats (FISCHER & REHÁK 2010, CHMELAŘ 2014, REHÁK et al. 2022), mostly apparently by the displacement of the smaller *Lacerta agilis* from the optimal habitats for *L. viridis.* As a result of climate warming, this phenomenon may become increasingly important. The Czech populations of *L. viridis* are more resistant to longer dry periods and rising temperatures caused by the climatic change. *L. agilis* is generally better equipped to inhabit relatively colder and wetter habitats (ENRIQUEZ-URZELAI et al. 2022). The large overlap of areas with a high probability of occurrence of both species should be interpreted that these habitats are suitable for both species, but are not equally used by both species due to their competition. In colder and wetter habitats, interactions with the viviparous lizard, *Zootoca vivipara* (LICHTENSTEIN, 1823), may occur. With the increasing number of records of *Podarcis muralis* in the Czech Republic (JABLONSKI et al. 2019, VLČEK & ZAVADIL 2019), it is possible to consider interactions with this species in the future as well. It is known that in the syntopic occurrence of *L. agilis* and *P. muralis,* both species can overlap in both space and niche usage (HEYM et al. 2013), but can result in a niche separation within the same habitat (FRÜHLING et al. 2022).

However, anthropogenic activities seem to be the largest danger to the *L. agilis* populations in the Czech Republic. In general and in agreement with others (MORAVEC 2015, MORAVEC 2019, MIKÁTOVÁ & JEŘÁBKOVÁ 2023), we consider the most serious cause of the decline of *Lacerta agilis* to be the homogenization of the landscape, and the dramatic decline of suitable habitats occurred as a result of the creation of large agricultural areas and urbanized areas. The removal of trees and shrubs opens up large areas for the pioneer colonisation by *L. agilis* and these areas are later ecologically degraded so that they act as an "ecological trap". The preferred *L. agilis* habitats contain high number of open spots, high percentage of grass and small bush coverage (see also NEMES et al. 2006, ČEIRĀNS 2007, MIZSEI et al. 2023). Patches with dense vegetation provide needed shelter from predation. Large newly cut open areas often do not contain enough solitary shrubs and other potential shelters from predators. Synanthropic species like cats, dogs and corvid birds prey on *L. agilis* and other reptiles. Other major dangers are habitat fragmentation and overgrowing. Dense coverage of trees and shrubs limits thermoregulation and can make previous presence sites uninhabitable for the species. The habitats preferred by *L. agilis* seem to be among the less resistant to climate change which poses a major threat to reptile populations (SINERVO et al. 2010, DUBEY et al. 2013).

The comparison of our predictive model and the real distribution shows that the predicted and real distributions are almost fully accordant. Predictive distribution models are a helpful instrument to facilitate monitoring and conservation efforts, especially when the populations of studied species decline at an alarming rate. We consider the large areas with high probability of occurrence identified by the model to be extraordinarily important, with need to direct species monitoring here. In our opinion, these sites should be considered as a matter of priority for conservation habitat management. Also, the focus should be on opening possible corridors for migration to maintain gene flow in otherwise increasingly isolated populations.

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Amphibia-Reptilia

One snake to eat them all: A predictive model of Coronella austriaca distribution in the Czech Republic and its comparison to syntopic reptile species

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Abstract:	The smooth snake, Coronella austriaca, is recorded from many areas of the Czech Republic state, but occurs mosaically in small isolated populations, which may be reflected in low genetic variability and high inbreeding. Its diet includes a significant representation of reptiles, so its occurrence is also intercorrelated with their availability. Due to its relatively high position in the trophic chain, its distribution is sensitively related not only to the distribution of hunted reptiles, but is also a remarkable bioindicator of the quality of the respective biotopes and natural communities. Conservation monitoring of its populations is complicated by its very inconspicuous way of life, due to which its presence often remains undetected. We created a predictive model and determined key factors influencing distribution of Coronella austriaca in the Czech Republic. The most relevant factors were: slope, annual precipitation and altitude. There are also obvious correlations of the distribution of Coronella austriaca with the presence of Lacerta agilis and Lacerta viridis. The model, which is finer than coarse grid maps and is less affected by differences in monitoring effort than spot maps, is in full accordance with the presence records and can be applied in the conservation of Coronella austriaca, e.g. identifying areas with preferred habitat to focus species monitoring and management efforts and opening of migration corridors. The model also suggests vulnerability of the identified habitats to climate change and anthropogenic activities. We consider the most serious risk to the Coronella austriaca in the Czech Republic to be the homogenization of the landscape and the dramatic decline of suitable habitats occurred as a result of the creation of large agricultural and urbanized areas.
Keywords:	Squamata, Colubridae, species conservation, habitat management, bioindication, climatic change
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One snake to eat them all: A predictive model of *Coronella austriaca* distribution in the Czech Republic and its comparison to syntopic reptile species

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Abstract.

The smooth snake, *Coronella austriaca*, is recorded from many areas of the Czech Republic state, but occurs mosaically in small isolated populations, which may be reflected in low genetic variability and high inbreeding. Its diet includes a significant representation of reptiles, so its occurrence is also intercorrelated with their availability. Due to its relatively high position in the trophic chain, its distribution is sensitively related not only to the distribution of hunted reptiles, but is also a remarkable bioindicator of the quality of the respective biotopes and natural communities. Conservation monitoring of its populations is complicated by its very inconspicuous way of life, due to which its presence often remains undetected. We created a predictive model and determined key factors influencing distribution of *Coronella austriaca* in the Czech Republic. The most relevant factors were: slope, annual precipitation and altitude. There are also obvious correlations of the distribution of *Coronella austriaca* with the presence of *Lacerta agilis* and *Lacerta viridis*. The model, which is finer than coarse grid maps and is less affected by differences in monitoring effort than spot maps, is in full accordance with the presence records and can be applied in the conservation of *Coronella austriaca*, e.g. identifying areas with preferred habitat to focus species monitoring and management efforts and opening of migration corridors. The model also suggests vulnerability of the identified habitats to climate change and anthropogenic activities. We consider the most serious risk to the *Coronella austriaca* in the Czech Republic to be the homogenization of the landscape and the dramatic decline of suitable habitats occurred as a result of the creation of large agricultural and urbanized areas.

Key words. Squamata, Colubridae, species conservation, habitat management, bioindication, climatic change.

Introduction

Predictive modelling of species distribution is a valuable tool in understanding specific requirements affecting it (Elith et al. 2006, Civiš 2013). Modelling for conservation purposes is becoming common in reptiles and amphibians (e.g. Santos et al. 2006, Kaliontzopoulou et al. 2008, Sillero & Carretero 2013, Oraie et al. 2014, Hosseinian Yousefkhani et al. 2015, Wirga and Majtyka 2015, Petrosyan et al. 2020, Chmelař et al. 2020, Srinivasulu et al. 2021, Chmelař et al. 2023, Kornilev et al. 2023), recently also in connection to the impact of climate change on species distribution (Carvalho et al. 2011, Dubey et al. 2013, Brito et al. 2014,) and spreading of invasive species (Pyron et al. 2008, Jarnevich et al. 2018). In the present-study on *Coronella austriaca* Laurenti, 1768 in the Czech Republic, we used the distribution model also to compare its habitat requirements to other selected syntopic reptile species which it preys upon.

The recent distribution of *Coronella austriaca* in Europe seems to be a result of several expansions from refugia in the Iberia, the Balkans, and Caucasus (Galarza et al. 2015, Jablonski et al. 2019, Stratakis et al. 2022) regions. Central European populations are mostly isolated from each other and have high inbreeding and low heterozygosity (Sztencel-Jabłonka et al. 2015) with the genetic variability decreasing even more in northernmost populations (Galarza et al. 2015). Viable isolated populations are known even from relatively very small localities (Dick and Mebert 2017). The whole Czech Republic is within the range of the species (Moravec 2019). *C. austriaca* can be found here dispersed in a number of small isolated populations within various suitable habitats, excluding high mountains, intensively used agricultural terrain and dense forests (Rehák 1992a, Moravec 2015, Mikátová and Jeřábková 2023). In northern parts of the species the species range, *C. austriaca* inhabits areas with a dense heath cover, e.g. in England (Spellerberg and Phelps 1977, Pernetta 2009, Reading 2012) and the Netherlands

(Stumpel and van der Werf 2012), even drained peat bogs in Latvia (Čeirāns and Nikolajeva 2014, 2017).

Legislative regulations in the Czech Republic list *C. austriaca* as Highly Endangered and Vulnerable according to the current Red List of Amphibians and Reptiles for the Czech Republic (Jeřábková et al. 2017). Published data of *C. austriaca* presence are available from most parts of the Czech Republic (Rehák 1992b, Vlašín 2001, Moravec 2015, Mikátová and Jeřábková 2023). Nevertheless, knowledge of its distribution and biology here remains small and fragmentary. The Nature Conservation Agency of the Czech Republic is monitoring occurrence of reptile species since 2007. However, conservation monitoring of *Coronella austriaca* still has many gaps, due to the fact that its presence is easily overlooked because of its inconspicuousness. At the same time, due to its position in the trophic chain, the occurrence of *Coronella austriaca* is also a useful bioindicator of the ecological status of the relevant biotopes and natural communities.

The aim of this paper is to present a model of the distribution of *Coronella austriaca* in the Czech Republic, compare it with models for some other selected reptile species, analyse available occurrence records to understand the isolation of individual populations, describe key climatic and geographic factors influencing the distribution of *C. austriaca*, identify areas with potential for dispersal of the species, and provide a tool for assessing how climate change may affect distribution.

Material and methods

We used a statewide database maintained by the Nature Conservation Agency of the Czech Republic as a source of *C. austriaca* presence sites. The database contained 1734 records from the whole territory of the Czech Republic collected within the period from 1906 to 2014.

We created 73 layers in total for the modelling purposes covering the area of the Czech Republic: the lowest, highest and average temperatures for individual months (36 layers in total), precipitation in individual months (12), bioclimatic variables according to worldclim.org methodology (Table 1) (19), altitude, surface exposure, human footprint, slope, road network, water courses and bodies including a 200 m buffer (Civiš 2013).

Tab. 1. Bioclimatic variables according to worldclim.org methodology.

Variable	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

WorldClim Worldwide database, which is routinely being used as the source of climate variables, only uses data from two meteorological stations for the whole Czech Republic (Hijmans et al. 2005), this made it unsuitable for our research and we have created layers for the bioclimatic variables manually in ArcGis 9.3 (ESRI ArcGIS 2008) using the 2D coordinate system SJTSK Krovak East-North. The layers of climate variables were created, based on the Climate Atlas of Czechia (Czech Hydrometeorological Institute 2007), which includes data from 1961 to 2000.

Variables containing maximum, minimum and average temperatures and precipitation in individual months were not included in the model because of their high intercorrelation. In similar cases, a careful interpretation is recommended for making possible implications for species conservation (Syfert et al. 2013). These data were also already included in the WorldClim bioclimatic variables.

The climatic variables were screened for intercorrelation in ENM Tools (Warren et al. 2010), resulting in correlation matrices for the Pearson correlation coefficient "r", Pearson coefficient of determination "r " and Variance Inflation Factor "VIF" (Suppl. material 1).

Variables with r > 0.8, r > 0.8 and VIF > 10 were considered heavily intercorrelated (Pradhan 2016) and removed from the model not to be used alongside variables with which they were closely correlated. The final model included the following variables: altitude,
aspect, human footprint, road network, BIO 1, BIO 2, BIO 3, BIO 4, BIO 6, BIO 7, BIO 8,
BIO 9, BIO 12, BIO 15, slope, water bodies and courses.

The Predictive Distribution Model was created using MaxEnt software (Phillips et al. 2023), the output being a GIS document in .asc format. This software was specifically chosen to work well with presence-only data (Elith et al. 2006, Hernandez et al. 2006). As the *C. austriaca* a presence prediction value, the “Logistic threshold” was used to optimize between the sensitivity of the model and the location of all real presence records in the predicted areas. MaxEnt model was run in three replications and automatically cross-validated. All other settings in the model were set to default.

Results

The mean predictive strength of the model (AUC) was 0.735 (73.5% of actual presence records were above the prediction threshold). The mean "logistic threshold", when the sensitivity of the model is equal to its specificity, was 0.412. Higher value means that the model predicts *C. austriaca* presence (see Fig. 1). The most important contributors to the resulting model were: slope inclination (explaining 25.5 % variability), BIO 12 - annual precipitation (14.9 %), altitude (12.2 %), Water_buffer - water courses and bodies with 200m buffer (10.8 %) and BIO1 – mean annual temperatures (9.6 %). Response curves of the 3 most influential variables are shown in Figures 2 to 4.

According to the model, the probability of presence of *C. austriaca* is highest with the slope inclination of 20-30° (Fig. 2), in the areas with annual precipitation of 550 mm (Fig. 3), altitudes up to 400 m a.s.l. and dropping up to altitudes of 1400 m a.s.l. (Fig. 4), areas with average annual temperatures of 10-11° C, and in areas up to 200 m from water courses and bodies.

Fig. 1. Predicted distribution of the smooth snake lizard (*Coronella austriaca*) in the Czech Republic.

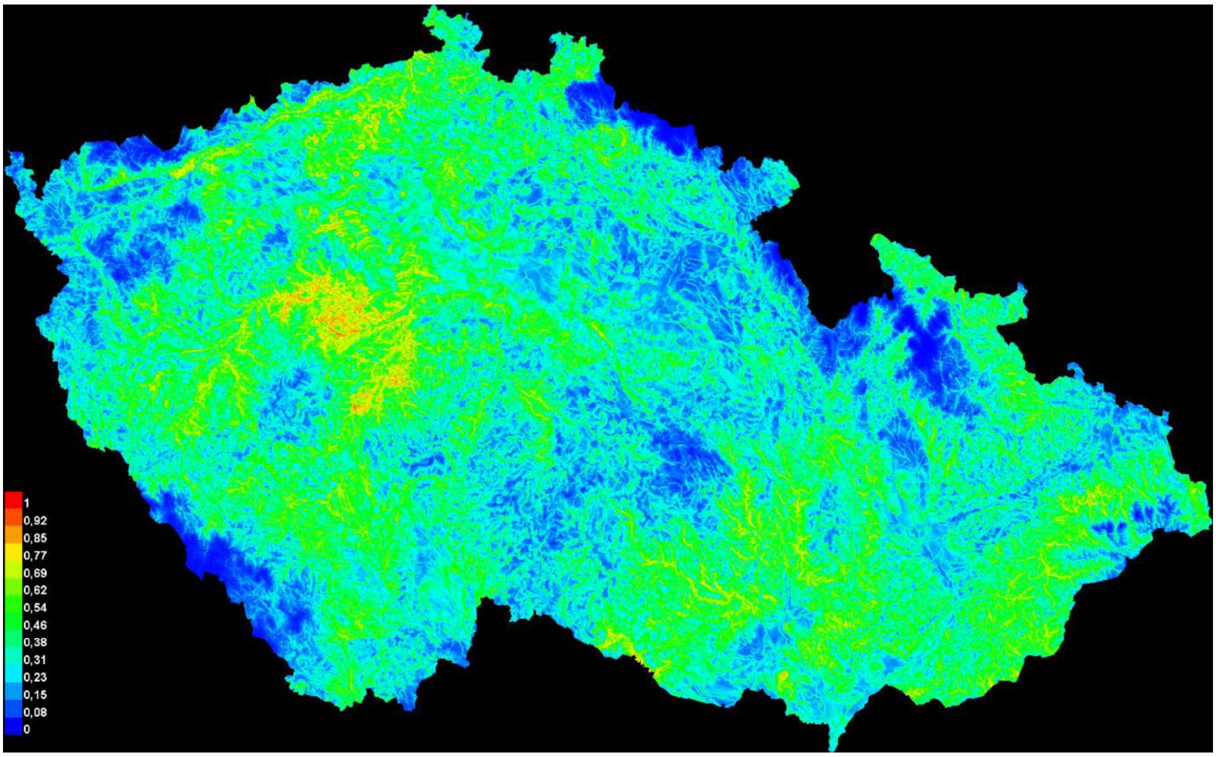

Fig. 2. Response curve of slope (°).

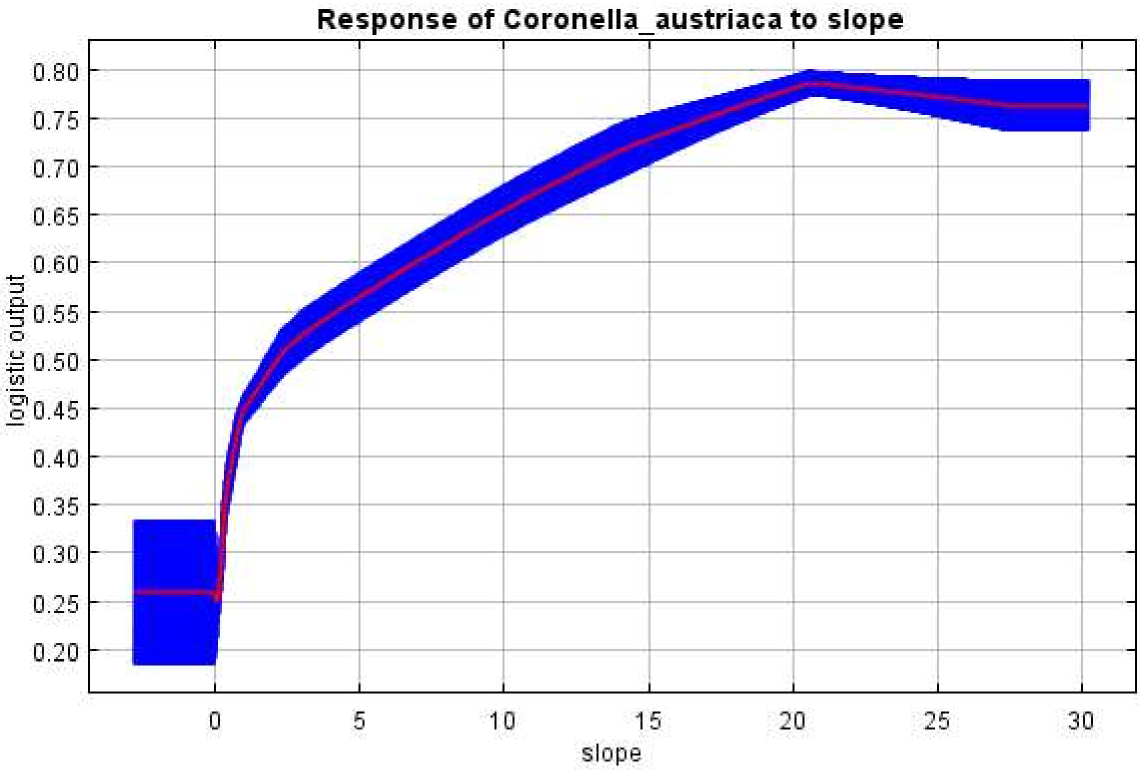

Fig. 3. Response curve of annual precipitation (mm).

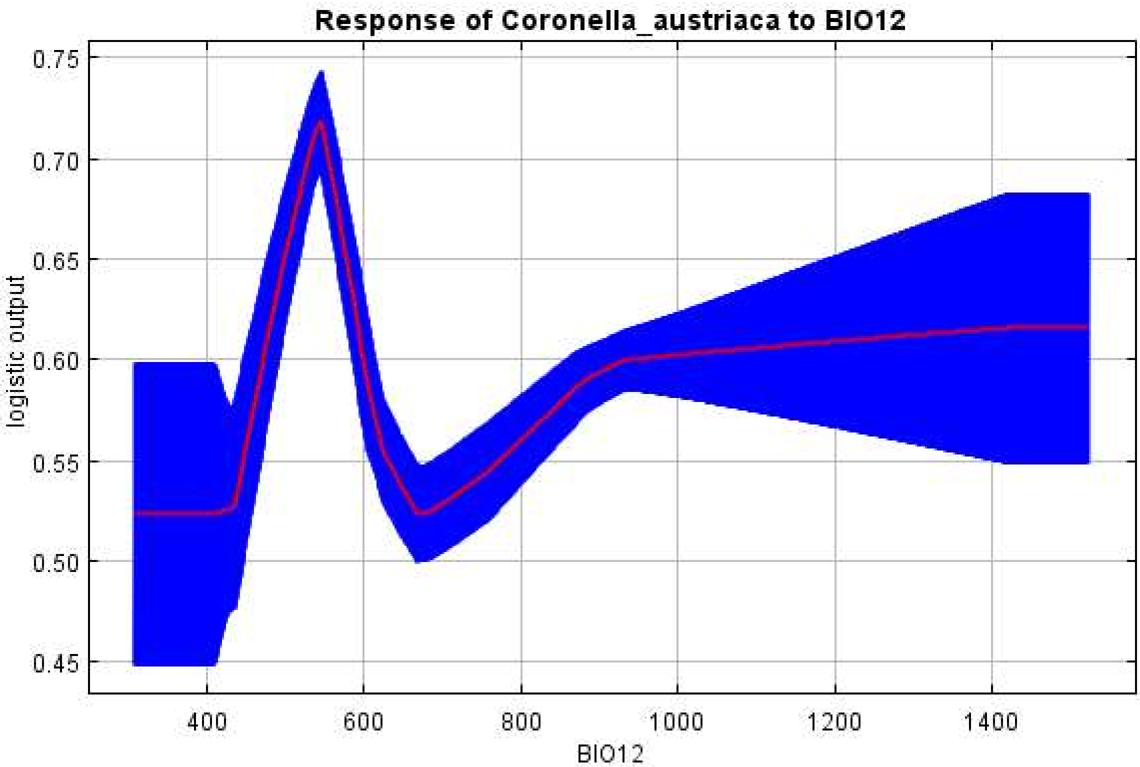

Fig. 4. Response curve of altitude (m a.s.l.).

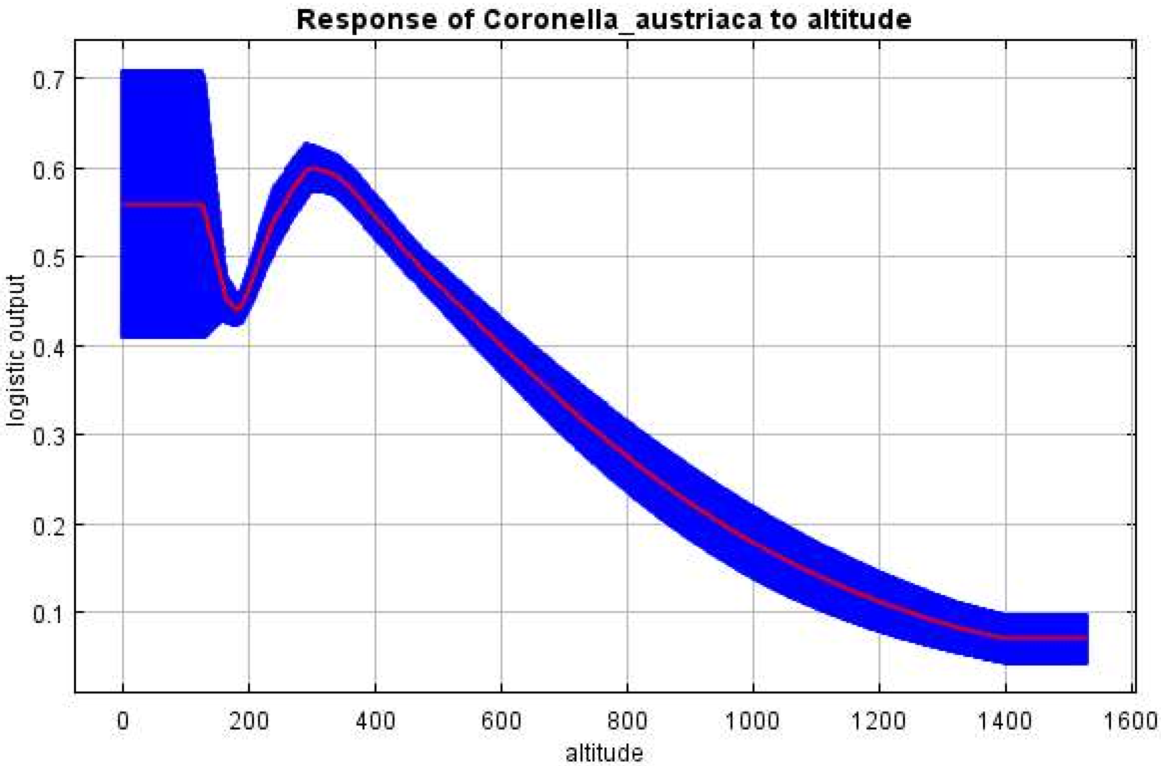

Fig. 5. Habitat of *Coronella austriaca* (maintained by active management). Roztocký háj-Tiché údolí Nature Reserve, Czech Republic.

Fig. 6. *Coronella austriaca* at the Zadní Bahna locality, Brdy Protected Landscape Area, Czech Republic.

Discussion and conclusions

The predicted and recorded distributions are almost fully accordant. The predictive strength of the model was moderate (mean AUC = 0.735) with 26.5 % of the actual presence records situated below the predicted occurrence threshold, including several areas where *C. ausriaca* occurrence is unlikely. These records are marked as controversial in the source database and their number is negligible and we consider it a price for a large data sample from a wide spectrum of informants. Snake species and especially *C. austriaca* can be mistaken for one another, but input to the database is only open to biologists authorized by the Nature Conservation Agency of the Czech Republic so we consider included records to be reliable. Moreover, the large size of the input data set contributes to prediction accuracy (Hernandez et al. 2006, Merow et al. 2013). The moderate prediction strength of the model suggests a preference of climatically or geographically specific habitats, but not an extreme habitat specialisation, especially when compared to some other Czech reptile species. Distribution models for the European green lizard, *Lacerta viridis* (Laurenti, 1768) and the dice snake, *Natrix tessellata* (Laurenti, 1768) had very strong AUC values (> 0,9), supporting a close link to very specific habitats in the Czech Republic (Chmelař et al. 2020, 2023). On the other hand, our distribution model for, the sand lizard, *Lacerta agilis* Linnaeus 1758, a widespread reptile species in the Czech Republic, resulted in a very low AUC, showing the wide ecological valence of the species (unpublished).

The increasing probability of species presence with increasing slope inclination is in accordance with the published sources from Central European populations, the preferred habitat being described as rocky slopes with xerothermic grass and shrubs (Vlašín 2001, Rybacki 2008, Moravec 2015, Mikátová and Jeřábková 2023). Slope inclination also increases the prediction probability of *Lacerta viridis* presence in the Czech Republic (Chmelař et al 2020), which *C. austriaca* preys upon. Strong influence of slope on the species distribution is also reported from the Iberian Peninsula (Santos et al. 2009). Major part of secondary anthropogenically created habitats of *C. austriaca* are also located in slopes, e.g. stone quarries, sandpits, railway and road embankments and rocky terrasses of vineyards and orchards (Rehák 1992a, Vlašín 2001, Moravec 2015, Mikátová and Jeřábková 2023).

The annual precipitation seems to affect the species distribution with a peak in approximately 550 mm per year, but shows no avoidance of even excessively humid locations with the probability of prediction decreasing only mildly with increasing precipitation. This is in accordance with published data from the Czech Republic, mentioning occurrences on river banks and in humid clearings (Moravec 2015, Mikátová and Jeřábková 2023). Data from the isolated populations in the southern part of the species range confirm preference of habitats with high slope, high precipitation and lower temperatures (Santos et al. 2009). *C. austriaca* is probably able to compensate behaviorally for longer humid periods by lowering their activity. There is also a strong assumption of link of average annual precipitation with average annual temperatures (Schultz and Halpert 1993), and while the highest probability of predicted presence is in temperatures of 10-11 °C, the probability does not drop sharply until reaching 7 °C. The species can partly compensate excessively warm periods by altering its diurnal activity (De Bont et al. 1986, Moravec 2015, Kolanek and Bury 2021). We also presume that viviparity is an effective adaptation for occurrence and reproduction in areas with suboptimal conditions. The average and maximum temperatures on the majority of the area are gradually increasing from 1961 (Zahradníček et al. 2020). The increase seems to be even faster in years 2011 to 2019 and these increases are most severe in the already warmest areas of the Czech Republic with the increase as high as 0.43°C per 10 years. This could mean significant future changes of suitable habitats for many species, including *C. austriaca* and its prey (see also Santos et al. 2008).

The effect of altitude on the species distribution was an expected result since the probability of *Lacerta agilis*, a common prey of *C. austriaca* in the Czech Republic (Brück 1965, Král et al 1983, Vogel 1984, Zavadil and Šapovaliv 1990, Moravec 2015, Mikátová and Jeřábková 2023), is also dependent on altitude and is highest in the altitudes up to 400 m a.s.l. as shown by our predictive model for this species (unpublished). This corresponds with the model, where the probability of occurrence of *C. austriaca* is highest in altitudes up to 350 m a.s.l. and gradually drops up to 1400 m a.s.l. The highest recorded presence of *C. austriaca* in the Czech Republic is 840 m a.s.l. (Vlašín 2001), but in Western and Central Europe, *C. austriaca* can be found up to altitudes of 1763 m a.s.l. (Grillitsch and Cabela 2001).

The most recently published maps of distribution of C. *austriaca* in the Czech Republic (Moravec 2015, Mikátová and Jeřábková 2023, Šandera 2024) are in good consistency with our predictive model, but these are square network maps where dimensions of one square are is 10 x 10 km which makes a detailed comparison problematic since the squares of this size often include areas where the species is not present. This method is also not suitable to study population ecology since even one presence record is enough to cover the whole square. The latest atlas of reptile distribution in the Czech Republic (Mikátová and Jeřábková 2023) also includes a map of localities of species occurrence which is more comparable with the model. The advantage of the model is that it is finer than coarse grid maps and is less affected by differences in monitoring effort than spot maps and by the fact that mapping of elusive species like *C. austriaca* faces problems with their generally low detectability, which can also significantly differ both seasonally and between consecutive years (Kolanek and Bury 2021). According to our model, *C. austriaca* can occur in most of the territory of the Czech Republic and even though it is recorded (at least historically) from over 60 % of quadrates covering the Czech Republic, its distribution is fragmented into small isolated populations (Moravec 2015, Mikátová and Jeřábková 2023), which can result in low genetic variability and high inbreeding (Galarza et al. 2015, Sztencel-Jabłonka et al. 2015, Kolanek et al. 2017).

We expect the occurrence of *C. austriaca* to be linked to the presence of other reptile species that are its prey. The present model of the distribution of *C. austriaca* shows that suitable conditions for its presence in the Czech Republic are also suitable for *L. agilis* (own data) and shares hotspots of high prediction probability with stenotopic *Lacerta viridis* (Chmelař et al. 2020) and *Natrix tessellata* (Chmelař et al. 2023). New sources of prey are also opening for *C. austriaca* with the increasing number of records of *Podarcis murali*s (Laurenti, 1768) in the Czech Republic (Jablonski et al. 2019, Vlček & Zavadil 2019), and a recent colonisation by *Podarcis tauricus* (Georgi, 1801) (Fischer et al. 2019, Rehák et al. 2022). In recent years, Czech populations of *L. agilis*, the common prey of *C austriaca*, seem to be gradually spreading to higher altitudes (Mikátová and Jeřábková 2023). The dispersal abilities of *C. austriaca* are much lower (Sztencel-Jabłonka et al. 2015) and therefore we see an urgent need to study-the response of this species. Of course, in this case it should be mentioned that *C. austriaca* has a wider food spectrum. In the Czech Republic, suitable alternative food for *C. austriaca* are also the widely distributed slow worms, *Anguis fragilis* Linnaeus, 1758 and *Anguis colchica* (Nordmann, 1840), which can even fully replace lacertids as a food source (Rehák 1992a). Czech *L. agilis* populations are rapidly declining in recent years (Moravec 2015, Mikátová and Jeřábková 2023) and even though no population of *C. austriaca* has been observed due to decline of the prey population (see also Reading and Jofré 2020), there may be a certain threshold in place. The literature states no clear signs of competition between *C austriaca* and other sympatric snake species, often even sharing microhabitat for basking (Zdunek and Jarmoliński 2023) and wintering (Hromádka and Voženílek 1976) sites. Despite this common coexistence, *C. austriaca* has been observed to prey on other snake species (e.g. Rehák 1992a).

According to our opinion, the largest danger to *C. austriaca* populations in the Czech Republic are anthropogenic activities resulting in the homogenization of the landscape as result of the creation of large agricultural areas and urbanized areas. Synanthropic species like cats, dogs and corvid birds prey on *C. austriaca* and other reptiles.

Populations of *C. austriaca* are often located in the immediate vicinity of roads which provides suitable habitat, but also poses a risk of road mortality (Ławicki et al. 2011). Since the secondary anthropogenic sites are highly used (Rehák 1992a, Vlašín 2001, Najbar 2006, Moravec 2015, Mikátová and Jeřábková 2023), the risk of their degradation is a significant problem for *C. austriac*a populations. Other major dangers are habitat fragmentation and overgrowing. Dense coverage of trees and shrubs limits thermoregulation and can make previous presence sites uninhabitable for the species and its prey. The habitats preferred by *C. austriaca* are also affected by the climate change, especially by rising temperatures and decreasing precipitation (Santos et al. 2008), which poses a major threat to reptile populations (Sinervo et al. 2010, Dubey et al. 2013).

Predictive distribution models are a helpful instrument to facilitate monitoring and conservation efforts, especially when the populations or habitats of studied species decline at an alarming rate. Large areas with high probability of occurrence identified by the model are in our opinion extraordinarily important and should be considered as a matter of priority for species and habitat conservation management. Predictive modelling for *Coronella austriaca*, due to its bioindicative value, given its position in the trophic chain and distribution sensitively related to availability of preyed reptiles, also provides a useful tool for assessing the quality of the natural habitats and natural communities. Considering the mosaic distribution, isolation of populations from each other and low genetic variability and inbreeding of *Coronella austriaca* in Central Europe, there is an urgent and strong need to focus also on the opening and maintenance of corridors allowing migration to enable genetic flow.

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Supplementary File

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Suppl. material 1 - Variable correlation matrix.xlsx

Příloha č. 2

Publikace, které nejsou součástí disertační práce:

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Variability of colour pattern and genetic diversity of Salamandra salamandra (Caudata: Salamandridae) in the Czech Republic

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RESEARCH PAPER

Variability of colour pattern and genetic diversity of *Salamandra salamandra* (Caudata: Salamandridae) in the Czech Republic

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Abstract. Two evolutionary lineages of the fire salamander occur in central Europe: the typically striped subspecies *Salamandra salamandra terrestris* (Bonnaterre, 1789) and the typically spotted *Salamandra salamandra salamandra* (Linnaeus, 1758). In the Czech Republic, fire salamanders have traditionally been viewed as belonging to the *S. s. salamandra* evolutionary lineage. Nevertheless, the colour pattern of some individuals in the westernmost part of the Czech Republic resembles that of *S. s. terrestris* in having parallel continuous bands along the back. In this study, we investigated whether in the Czech Republic the presence of striped fire salamander phenotype could be associated with the genotype of *S. s. terrestris*. We sequenced the mitochondrial D-loop and two nuclear markers, Rag2 and PDGFRα, of 61 fire salamander individuals from the Czech Republic. To describe the geographical distribution pattern of the striped and spotted fire salamander phenotype in the Czech Republic, we evaluated colour phenotypes of 398 individuals from ten localities distributed so as to cover the whole country. We found no evidence of presence of genotypes corresponding to the *S. s. terrestris* lineage. We did, however, find that the striped phenotype is found mostly in the northwest of the Czech Republic, where both the striped and the intermediate phenotype occur significantly more frequently than in the rest of the country, where the spotted phenotype seems dominant. This finding indicates that Czech and Polish populations of *S. salamandra* show a degree of phenotypic pattern variation comparable to that observed in German populations, although at a local level the frequencies of the striped and spotted phenotype vary. It would be interesting to test whether a genetic toolkit responsible for the colour pattern is shared via genetic introgression between populations, or whether the striped phenotype of Czech fire salamanders evolved independently.

Key words: fire salamander, *S. s. terrestris*, coloration, pigment, evolution, biogeography, Bohemian Massif, Central Europe

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Introduction

Coloration can aid animal survival and reproduction in various ways, including protection against solar radiation, crypsis, communication, and recognition of conspecifics (Cuthill et al. 2017). One evolutionarily important function of body coloration is its role in speciation (Andersson & Simmons 2006). For instance, once females develop preference for a particular colour patterns in males, coloration can – much like geographical isolation – function as a prezygotic reproductive barrier (Kirkpatrick & Ravigné 2002). On the other hand, the genetic toolkits responsible for colour and colour patterns can be shared via hybridisation bewtween distinct evolutionary lineages (Dasmahapatra et al. 2012, Taylor & Larson 2019) and can also lead to speciation (Mallet 2007). Identification of colour phenotypes in closely related evolutionary lineages is therefore important in understanding the functional role of coloration in a species.

Although fire salamander (*Salamandra salamandra* Linnaeus, 1758) coloration is limited to brown, black, red, and yellow, different lineages of fire salamanders display a range of distinct colour patterns (Seidel & Gerhardt 2016). Fire salamanders develop their colour pattern, i.e. the form and arrangement of spots and stripes, after metamorphosis (Pederzoli et al. 2003). During the course of their life the colour pattern of fire salamanders from the Balkan peninsula undergoes only subtle changes (Wisniewski & Wisniewski 1998), but some pattern changes have been reported in Slovak populations (Balogová et al. 2016) and the Corsican fire salamander (*Salamandra corsica* Savi, 1838) exhibits pronounced colour pattern changes (Beukema 2011). The colour pattern of fire salamanders in the Czech Republic has been used by researchers to distinguish individuals (Opatrný 1983, Peprný 2000).

In central Europe, we find two evolutionary lineages of *S. salamandra*, which have traditionally been considered distinct subspecies (Veith 1992, Steinfartz et al. 2000, Dufresnes 2019). The banded fire salamander (*Salamandra salamandra terrestris* Bonnaterre, 1789), type locality Normandy (Eiselt 1958), is found from the Pyrenees to Germany. The spotted fire salamander (*S. s. salamandra* Linnaeus, 1758), type locality Nürnberg (Mertens & Müller 1928), is distributed throughout eastern Germany, central and eastern Europe, and the Balkans (Thiesmeier & Grossenbacher 2004, Seidel & Gerhardt 2016). As the vernacular names suggest, representatives of the two lineages differ in their dorsal colour pattern. *Salamandra s. terrestris* have two parallel stripes along their back, while spotted fire salamanders are characterised by an irregular

Fig. 1. Colour pattern phenotypes of fire salamanders (*Salamandra salamandra*) from the studied area. A) the striped phenotype from north-western Bohemia, Czech Republic. B) the spotted phenotype from central Bohemia, Czech Republic (photos Jiří Moravec).

distribution of isolated spots on their back (Boulenger 1911). Boulenger (1911) claimed that there are no transitions or intermediate phenotypes in-between the striped *S. s. terrestris* of western Europe and the spotted *S. s. salamandra* of eastern Europe. Later authors, however, demonstrated that intermediate phenotypes do exist and in fact occur widely in central Europe (Eiselt 1958, Steinfartz et al. 2000, Weitere et al. 2004). According to Arnold (2002), the two lineages share a contact zone, which – based on colour pattern phenotypes – appears to stretch from eastern Germany to the Czech Republic and Poland. To date, only a few studies have attempted to quantify the distribution of fire salamanders' colour phenotypes (Klewen 1985, Beukema et al. 2016, Najbar et al. 2018, Burgon et al. 2020) and even fewer have related it to the distribution of genetic lineages (Veith 1992, Beukema et al. 2016, Najbar et al. 2018, Burgon et al. 2020).

The localisation of a contact zone between the western and eastern evolutionary lineages of fire salamanders, traditionally referred to as *S. s. terrestris* and *S. s. salamandra*, in central Europe remains an open question (Veith 1992, Arnold 2002, Najbar et al. 2018). This is despite the fact Weitere et al. (2004) described two contact zones based on mitochondrial D-loop sequences: one in south-western Germany, the other in north-western Germany. The most recent genome-wide study confirmed the presence of two contact zones in Germany, one in the extreme south, the other in the north of the country, including the Elbe Valley (Burgon 2018, Supplementary Figure A4.6). The northern contact zone is not limited to north-western Germany, it extends across the north towards the northeast but the study did not include any sampling points further to the east so the full extent of the distribution remains unclear (Burgon 2018).

In the Czech Republic, fire salamanders have historically been believed to belong to the evolutionary lineage *S. s. salamandra*. On the other hand, the presence of *S. s. terrestris* has been reported in Germany, near the north-western border of Bohemia, especially in the vicinity of Meißen, Dresden, and Zittau (Baruš & Oliva 1992, Zöphel & Steffens 2002), and striped phenotypes were found over a hundred years ago around Liberec, which is again in the north-western part of Bohemia (Pražák 1898). Phenotypical characteristics of some individuals in the westernmost part of the Czech Republic resemble those of *S. s. terrestris* in that they have parallel, more or less continuous stripes along the back (Baruš & Oliva 1992, Benda 2015, Moravec 2019). In the rest of the country, the spotted phenotype seems dominant (Fig. 1).

Hitherto, we do not have the data on either the exact distribution of colour phenotypes or the genetic variation of *S. salamandra* in the Czech Republic. In this study, we report our findings on the distribution of dorsal colour pattern phenotypes and genetic diversity of fire salamanders in the Czech Republic. Specifically, we ask whether the presence of the striped phenotype could be associated with the genotype of *S. s. terrestris* in the Czech Republic.

Material and Methods

Analysis of distribution of colour pattern phenotypes

To describe the geographical distribution of the striped and spotted phenotypes of fire salamanders in the Czech Republic (current distribution of *S. salamandra* in the Czech Republic, Jeřábková & Zavadil 2020), we collected photographs of 398 individuals (National Museum Praha (NMP-P6V), voucher specimens and living individuals recorded in the field) from ten localities which cover the whole range of the country (Jablečno, Vaňov-Ústí nad Labem, Děčín, Roztoky, Praha-Troja, Kuroslepy, Velká nad Veličkou, Libavá; coordinates in Table 1, Fig. 2). Previous studies on fires salamanders in the Czech Republic showed that colour pattern can be used to discriminate individuals (Opatrný 1983, Peprný 2000), hence no individual marking was needed to avoid re-captures.

Eiselt (1958) divided the colour pattern phenotypes of *S. salamandra* into four categories: striped, striped-spotted, spotted-striped, and spotted. Klewen (1985) and Najbar et al. (2018) expanded this division by adding further categories. Because we were mostly interested in differences between the striped and the spotted phenotypes, we simplified the division and, based on photographs, sorted individual phenotypes into three colour pattern categories following Benda (2015) or Veith (1992). The categories were defined as follows: a) striped: a yellow dorsal pattern consisting of two continuous or mostly continuous dorsolateral bands (Fig. 1A), b) intermediate: a symmetric dorsal pattern consisting mostly of separate spots, some of which may be asymmetric, c) spotted: no obvious continuous symmetric bands can be discerned, the yellow dorsal pattern consists of irregular, randomly distributed spots (Fig. 1B), which can be accompanied by a single continuous asymmetric stripe along the body.

We calculated the ratio of each colour pattern category for each population and interpolated the values for the geographical space of the Czech Republic using the RCzechia package (Lacko 2020) and interpolation function *idw* in the *gstat* package (Pebesma 2004) in R software (R Core Team 2017). Then we compared the counts of colour pattern categories between all populations with Pearson's chi-squared test using chisq.test followed by a post hoc analysis based on the residuals of Pearson's chi-squared test for count data in the chisq.posthoc.test package (Ebbert 2019) in R.

Genetic analyses

Sampling (Fig. 3A, Table S1) was designed to cover the west-east latitudinal gradient of the fire salamander range in the Czech Republic, with focus on the north-western part of the country where individuals of the striped phenotype were previously reported. We collected 77 individuals of *S. salamandra*, 61 of whom were collected specifically for this study in the Czech Republic and four in Slovakia. Collection took place in 2017-2018. Samples were obtained from roadkill. Samples from the core area of distribution of *S. s. terrestris* (two individuals from Germany) were kindly provided by Professor Wolfgang Böhme from collections of the Zoologische Forschungsmuseum Alexander Koenig in Bonn (ZFMK). Samples from the core areas of distribution of *S. s. salamandra* (five samples from Greece-NHMC, three from Albania, one from Ukraine, and one from Slovenia) were obtained from roadkill collected during another study.

Small tissue samples were preserved in 96% molecular grade ethanol. DNA was extracted using the Tissue Genomic DNA Mini Kit (GT300, Geneaid) according to the manufacturer's instructions. To barcode the salamanders, we used three DNA markers: the mitochondrial D-loop and two nuclear markers, Rag2 and PDGFRα, because it has been reported that these are sufficiently variable to enable a comparison between closely related evolutionary lineages (Vences et al. 2014). We used L-PRO-ML and H-12S1-ML to amplify the D-loop, PDGFRa2F and PDGFRa2R-F primers to amplify the PDGFRα, and RAG2-SAL-F2 and RAG2-SAL-R1 to amplify Rag 2 (Vences et al. 2014). PCR was performed in a final volume of 25 µl. PCR conditions were as follows: 120 s at 94 °C for initial incubation; 39 cycles of 20 s at 94 °C, 50 s at 60 °C, 180 s at 72 °C, and a final extension for 10 min at 72 °C. This was followed by PCR product purification (Šanda et al. 2008). Sequencing was carried out by Macrogen Service Centre Europe (Amsterdam, Netherlands) using amplification primers.

From the GenBank database (Sayers et al. 2019), we downloaded the sequences of *Salamandra salamandra gallaica* (D-loop – KX094979.1, PDGFRα – KF645649, Rag2 – KF645724.1) and *Salamandra salamandra longirostris* (D-loop – KF645599.1,

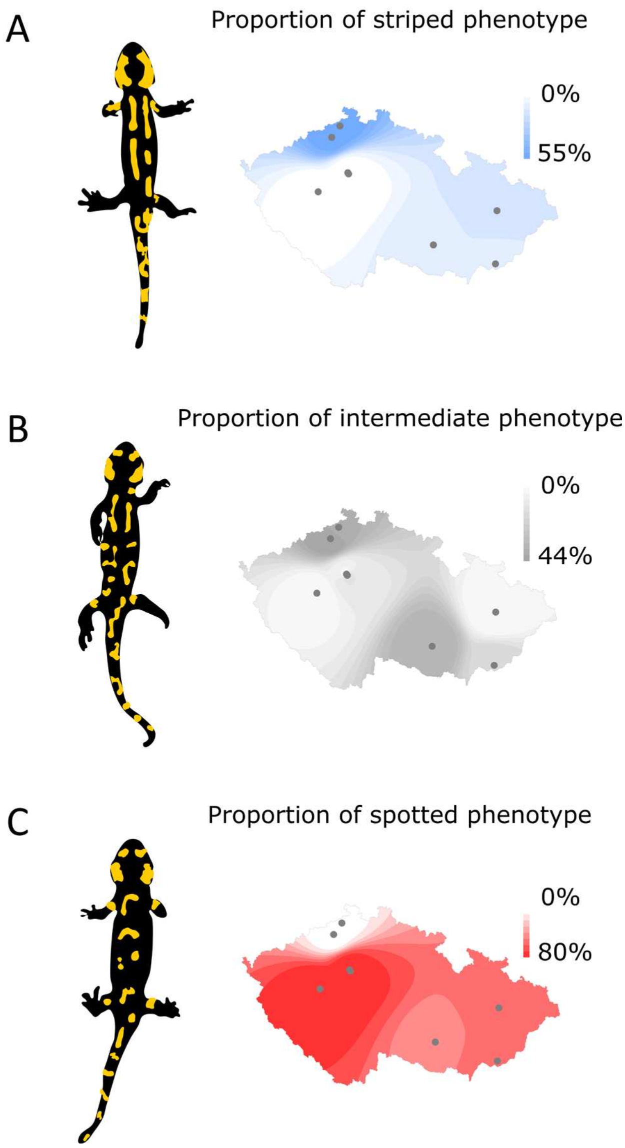

Fig. 2. The distribution of proportions of colour pattern phenotypes of fire salamanders in the Czech Republic as extrapolated in geographical space. A) striped phenotype, B) intermediate phenotype, C) spotted phenotype. Note that the scale for different phenotypes differs.

Table 1. Differences between populations and the rest of the Czech Republic after post hoc analysis based on residuals of Pearson's chi-squared test for counts of individuals belonging to one of the three colour pattern phenotypes.

Locality	Coordinates (WGS84)			Colour pattern		
	N	E	Value	Striped	Intermediate	Spotted
Jablečno	49.88	13.75	proportion of phenotype	0%	20%	80%
n = 10			*P* values	1	1	1
Ústí nad Labem-Vaňov	50.63	14.05	proportion of phenotype	52%	44%	4%
n = 25			*P* values	0	1	0
Děčín	50.79	14.22	proportion of phenotype	53%	40%	7%
n = 15			*P* values	0	1	< 0.001
Roztoky	50.15	14.39	proportion of phenotype	3%	19%	78%
n = 119			*P* values	0.080	0.060	< 0.001
Praha-Troja	50.13	14.4	proportion of phenotype	0%	30%	70%
n = 120			*P* values	< 0.001	1	0.346
Kuroslepy	49.15	16.21	proportion of phenotype	13%	40%	47%
n = 76			*P* values	1	0.720	0.233
Libavá	49.62	17.56	proportion of phenotype	20%	27%	53%
n = 15			*P* values	1	1	1
Velká nad Veličkou	48.89	17.53	proportion of phenotype	12%	29%	59%
n = 17			*P* values	1	1	1

PDGFRα – KF645651.1, Rag2 – KF645726.1) to use as outgroups when assessing the differentiation between *S. s. terrestris* and *S. s. salamandra*. All sequences were checked for quality manually and homologous regions were aligned using the ClustalW algorithm implemented in Geneious 9.0.5. (Kearse et al. 2012). Haplotypes of the mitochondrial D-loop were determined using DNAsp 5.10 (Librado & Rozas 2009) and the haplotype network was calculated in TCS 1.21 (Clement et al. 2002) using 95% connection limit and gap as the fifth character. The network was visualised using tcsBU (Múrias dos Santos et al. 2016). Sequences were concatenated and the neighbour joining tree calculated in Geneious 9.0.5 using the Juke-Cantor genetic distance model and bootstrap resampling (random seed = 467,663, with 10,000 replicates). The partition scheme was determined by Partitionfinder 2.1.1 (Lanfear et al. 2017). A Bayesian inference tree was calculated using MrBayes 3.2.6 (Ronquist et al. 2011). The analysis consisted of two simultaneous runs, four MCMC chains, and ten million generations. The trees were sampled every 100 generations, whereby the first 25% of trees were discarded. Bayesian posterior probabilities (BPP) were estimated from the post burn-in samples, and the 50% majority-rule consensus tree was generated from the retained posterior distribution trees.

Results

Analysis of distribution of colour pattern phenotypes

Table S2 presents a categorisation of individuals based on photographs. A distribution of ratios of colour pattern as well as interpolation of values onto the geographical space of the Czech Republic are shown in Fig. 2. Localities in the north-western part of the country (Děčín and Ústí nad Labem-Vaňov) clearly show an increase in the presence of striped and intermediate individuals and a

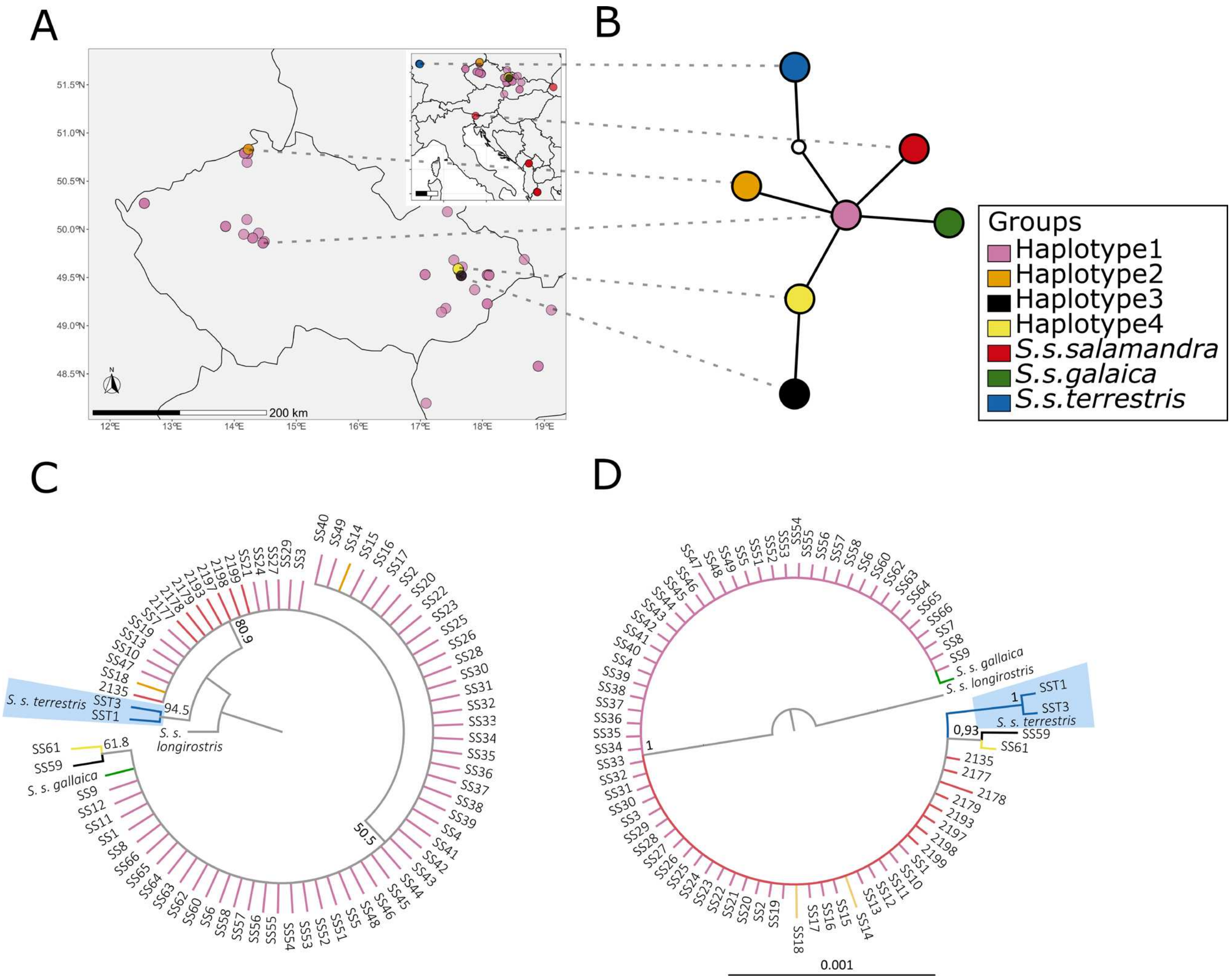

Fig. 3. Sampling design and the results of genetic analyses. A) Sampling design, where circles represent individuals and colour coding represents haplotype membership: blue – *Salamandra salamandra terrestris* from Germany, red – *Salamandra salamandra salamandra* from the Balkan peninsula and Ukraine, reddish purple – fire salamanders from the Czech Republic and Slovakia that bear "haplotype 1", the most common found in this study, orange – two individuals from Děčín (SS14, SS18) that bear "haplotype 2", black – individual SS59 from Oderské vrchy that bear "haplotype 3", yellow – individual SS61 that bears "haplotype 4". B) A statistical parsimony haplotype network (connection limit 95%) for 699 bp partial D-loop sequence of studied salamanders calculated using the TCS algorithm. In this network, branches represent mutations, small blank circles represent missing hypothetical haplotypes. C) Neighbour joining tree based on a concatenated sequence of mitochondrial D-loop and nuclear Rag2 and PDGFRα sequences (1,335 bp), where node numbers show consensus percentage support, while branches are of equal length. Individual samples are colour coded according to haplotype membership. D) Bayesian inference tree based on a concatenated sequence of mitochondrial D-loop and nuclear Rag2 and PDGFRα sequences (1,335 bp). Node numbers show probability, the scale bar represents the number of nucleotide substitutions per site, individual samples are colour coded by haplotype membership.

decrease in the occurrence of spotted individuals compared to the rest of the country. The presence of the intermediate phenotype was also increased at locality Kuroslepy and slightly elevated in Velká nad Veličkou and Praha-Troja. The highest occurrence of the spotted phenotype was observed in Jablečno, Praha-Troja, and Roztoky in the central part of Bohemia, whereby two of these localities, namely Jablečno and Roztoky, also had the lowest recorded presence of the striped and the intermediate phenotype.

The counts of individuals belonging to individual colour pattern categories differed significantly between populations χ^2 (df = 14, n = 398) = 133.41, $P < 0.001$. Results of a post hoc analysis based on the residuals of Pearson's chi-squared test for count data are summarised in Table 1. Our results confirm that localities in the north-western part of the Czech Republic (Děčín and Ústí nad Labem-Vaňov) differ significantly in the counts of both striped and spotted colour pattern phenotypes from the rest of the country (all $P < 0.001$). At these localities there is a high proportion of striped individuals. On the other hand, the Praha-Troja locality differs significantly from the rest of the country ($P < 0.001$) as there are no striped individuals. The Roztoky locality differs significantly in the count of spotted individuals

($P < 0.001$). We can thus conclude that localities in the north-western part of the Czech Republic harbour a higher proportion of the striped phenotype of *S. salamandra* than the rest of the country.

Genetic analyses

The sequencing and postprocessing of sequences resulted in a concatenated alignment of total length 1,335 bp (D-loop – 699 bp, GenBank accession numbers MZ436191-MZ436263; PDGFR*α* – 404 bp, GenBank accession numbers MZ436264-MZ436336; Rag2 – 232 bp, GenBank accession numbers MZ436337-MZ436397). We identified 11 single-nucleotide mutations in the D-loop region only one of which was parsimony-informative; the rest were singletons. Once we took the gaps into account, both the DNAsp and TCS identified eight mitochondrial haplotypes. One haplotype, represented by *S. s. longirostris* (*S. longirostris* sensu Frost 2020), turned out to be unconnected to the haplotype network because the probability of its connection to the network did not reach the 95% connection limit. One haplotype, represented by *S. s. terrestris*, was clearly separate from a group formed by the haplotype consisting of *S. s. salamandra* samples from eastern and south-eastern Europe. Then we also found a haplotype represented by *S. s. gallaica* and four haplotypes represented by individuals from the Czech Republic and Slovakia. One of these four haplotypes contained individuals from the north-western region of the Czech Republic (haplotype 2, locality Ludvíkovice), two haplotypes were represented by one individual each (both of which came from northern Moravia, namely haplotype 3, Libavá and haplotype 4, Týn nad Bečvou). All remaining individuals from the Czech Republic and Slovakia turned out to belong to the remaining fourth haplotype (haplotype 1, haplotype network in Fig. 3B).

We found only one single-nucleotide mutation in the partial PDGFR*α* sequence and two single-nucleotide mutations in the partial Rag2 sequence, but only one of those was parsimony-informative. The single-nucleotide mutation in the partial PDGFR*α* sequence was shared by the two individuals from the localities in Germany. The parsimony informative single-nucleotide mutation in Rag2 was shared by seven individuals sequenced in this study (the two individuals from Germany, four individuals from north-west of the Czech Republic, one individual from the central part of the country and one individual from the Czech-Moravian highlands) and sequence of *S. s. longirostris* downloaded from GeneBank. Both neighbour joining (Fig. 3C) and Bayesian inference-based (Fig. 3D) trees based on these concatenated data revealed a clear separation between individuals of *S. s. terrestris* and the rest of the samples studied. We therefore conclude that individuals from the Czech Republic included in our study do not belong to the *S. s. terrestris* lineage.

Discussion

We found that the striped phenotype of *S. salamandra* is most abundant in the north-western part of the Czech Republic (Fig. 2), where frequencies of the striped and intermediate phenotype differ significantly from the rest of the country (Table 1). Our finding supports and elaborates on previous reports according to which both fire salamander phenotypes are sympatric at least in a small part of the Czech Republic (Benda 2015, Moravec 2019; Fig. 1). Our results thus provoke questions regarding the historical evolutionary mechanisms which lead to the coexistence of the striped and spotted salamander phenotypes in the Czech Republic.

Different authors mention the varying distribution of the three phenotypes (striped, intermediate, and spotted) throughout Germany. The distribution of colour pattern phenotypes of *S. salamandra* in central Europe has been studied quantitatively in Germany by Klewen (1985) and in Poland by Najbar et al. (2018). In Leiberg (Germany, approximately 270 km west of the Czech Republic), most fire salamanders belong to the striped phenotype (80%), with spotted individuals making up only a small proportion of the population (< 1%) (Klewen 1985). This shows that the striped phenotype is characteristic of an area west of the Czech Republic. It has recently been reported that in eastern Upper Lusatia, the westernmost part of Poland – which is likewise adjacent to the German border (the locality is approximately 20 km from German Zittau) – there is a higher frequency of both the striped (15%) and the intermediate phenotype (57.9%) than in other Polish fire salamander populations (Najbar et al. 2018). Our data show that both the striped and the intermediate phenotype occur with the highest frequency at localities in the furthest north-west of Bohemia, Děčín (striped 53%, intermediate 40%) and Ústí nad Labem-Vaňov (striped 52%, intermediate 44%). These localities are in areas adjacent to the German border (e.g. Děčín is approximately 50 km from Zittau) in the Elbe Valley

(Fig. 2A, B). Benda (2015) showed that the striped phenotype occurs with a higher frequency (48%) than either the intermediate (22%) or the spotted form (30%) in Saxon-Bohemian Switzerland, Děčín highlands, which is similarly in the north-western part of the country, close to the German border. These results indicate that both the Czech and Polish populations of *S. salamandra* show a degree of phenotypic pattern variation comparable to that observed in the German populations, although the frequencies of the striped and spotted phenotype differ locally.

Within the Czech Republic, localities in north-western Bohemia are characterised by a high frequency of both the striped and the intermediate phenotype. In central Bohemia, we can observe a sort of hiatus, where the striped phenotype is virtually missing (< 5%) and the spotted phenotype reaches its maximum frequency (> 70%). In the eastern part of the country (Libavá), both the striped and the intermediate phenotypes are again found at slightly increased frequencies (20% and 27% respectively; compare Fig. 2A, C). This geographical trend is similar to that observed in the Polish populations, where after low occurrence in the western populations between Lusatia and Opawskie Mountains, the striped phenotype increases in frequency in the Opawskie Mountains (8%) (Najbar et al. 2018). The distance between the Czech Libavá and Polish Opawskie Mountains is less than 80 km, which means it is possible that the *Salamandra* populations of these regions are related. Our results did not, however, show a statistically significant difference between Libavá and other populations in the Czech Republic. It is thus also possible that the difference in frequency of the colour pattern phenotypes merely reflects naturally occurring random variation. Variability of colour pattern phenotypes in populations of the usually spotted *S. s. salamandra* remains virtually unknown. Future studies should attempt to quantify the abundance of colour pattern phenotypes in fire salamanders throughout their range to test the adaptive significance of colour patterns.

We found no evidence of the presence of genotypes corresponding to the *S. s. terrestris* lineage on Czech territory (Fig. 3) (for comparison between haplotypes presented in this study and haplotypes published on GenBank; Fig. S1), although *S. s. terrestris* and *S. s. salamandra* do share a contact zone in central Europe (Veith 1992, Steinfartz et al. 2000, Thiesmeier & Grossenbacher 2004, Weitere et al. 2004, Burgon 2018). Given that the contact zone between *S. s. terrestris* and *S. s. salamandra* is located at some distance from the Czech Republic any expectation of the presence of *S. s. terrestris* in the country might be unfounded. Nevertheless, estimates regarding the position of the contact zone have changed significantly over time (e.g. Freytag 1955, Gauckler 1980, Klewen 1991, reviewed in Veith 1992). Some relatively recent studies localise the contact zone only to eastern Germany or even to eastern France, Switzerland, or southern Austria (Veith 1992, Thiesmeier & Grossenbacher 2004). Weitere et al. (2004) concluded that there are two contact zones between *S. s. terrestris* and *S. s. salamandra*: one in south-western Germany, restricted to the vicinity of Karlsruhe, the other in north-western Germany, spanning several hundred kilometres from Ahaus to the Deister, near Hannover. The most recent genome-wide study suggests the presence of a lineage that shares its evolutionary history with individuals from France (i.e. individuals that would have traditionally been considered *S. s. terrestris*) in the very south of Germany and another lineage in the north, including the Elbe Valley (Burgon 2018, Supplementary Figure A4.6). Based on this high-throughput data, the putative contact zone thus would not have been limited to the north-western parts of Germany: it would extend across to the northeast. It remains unclear whether the distribution of the *S. s. terrestris* lineage reaches the very east of Germany, which may be possible given the presence of this lineage in the Elbe Valley in the north. However, at this point it must be noted that not only the distribution of central European lineages but also their subspecific status is a matter of discussion (Burgon 2018, Burgon et al. 2021).

An interesting question to be answered in the future is whether the colour pattern phenotypes are the result of shared or independent evolution of the German and Czech fire salamanders. The genetic toolkits responsible for the colour pattern of *S. s. terrestris* may be shared by genetic introgression from *S. s. terrestris* to Czech salamanders with the striped phenotype, which is a process known in other organisms (e.g. Zhang et al. 2016, Dannemann & Kelso 2017, Andrade et al. 2019). This scenario could be the case although the loci analysed in this study are not shared between the Czech fire salamanders and the *S. s. terrestris* lineage and the geographical distance between the non-admixed populations is rather long. On the other hand, the localities characteristic by an increased ratio of striped individuals are also characterised by presence of private haplotypes (Děčín – "haplotype 2", Fig. 3A, B; and Libavá and Týn nad Bečvou – haplotypes 3 and 4, Fig. 3A, B). This finding might suggest that these salamander populations have a more pronounced structure than those in the rest of the Czech Republic. It is possible that at these localities the striped phenotype evolved independently of the striped phenotype of *S. s. terrestris*. This is because 1) according to our results (Fig. 3C, D) the Czech salamanders and *S. s. terrestris* do not share any recent evolutionary history and 2) increased genetic structuring at these localities may have arisen via selection on colour pattern, as is the case in the Iberian fire salamanders (Burgon et al. 2020). The polymorphic Iberian fire salamander populations do not, however, exhibit a neutral genetic structure. This observation may imply the possibility that the Czech polymorphic populations are more ancient. However, we do not know whether any haplotype is specific for any colour pattern phenotype, because we did not examine the colour patterns of individuals that were used for genetic sampling. More detailed sampling, including reference individuals from Germany and Poland, would be needed to place our findings in a broader context of genetic variability of fire salamanders in central Europe. Moreover, different molecular techniques – such as nuclear microsatellite data or genome-wide next-generation sequencing – should be employed to improve the description of genetic variability of fire salamanders in central Europe.

Conclusions

Our data indicate that the striped phenotype of *S. salamandra* occurs more frequently in the north-western regions of the Czech Republic, which are closer to the contact zone with *S. s. terrestris*. Nevertheless, we found no evidence for the presence of genotypes corresponding to *S. s. terrestris* in Czech *Salamandra* populations. More research on the distribution of fire salamander colour patterns may reveal interesting facts about the distribution of their distinct evolutionary lineages and their evolutionary history, and perhaps also demonstrate some more general rules of evolutionary genetics.

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Supplementary online material

Fig. S1. Consensus neighbour joining tree (bootstrap-resampled, random seed = 237534, 10,000 replicates; reconstructed using Geneious 9.0.5, Biomatter Ltd.) of D-loop haplotypes, node numbers show consensus percent support, branches show nucleotide substitutions per site. 2,135 (Balkan and Ukraine individuals, red), SST1(Germany, *Salamandra salamandra terrestris*), SS1 (Czech and Slovak widespread "haplotype 1", purple), SS14 ("haplotype 2" from localities at Děčín, Czech Republic, orange), SS59 ("haplotype 3" from locality at Oderské vrchy, Czech Republic, black), SS61 ("haplotype 4" from locality at Lipník nad Bečvou, Czech Republic, yellow) represent haplotypes of samples sequenced in this study that were determined using DnaSP 5.10 (Rozas et al. 2010, Universitat de Barcelona); KY055013.1 and KY055014.1 represent haplotypes from Sudetes and Carpathians respectively in Poland determined by Konowalik et al. (2016); KT3359XX.X represent clade C haplotypes throughout of the Germany determined by Steinfartz et al. (2000); KX9519XX.X represent haplotypes from the Carpathians determined by Vörös et al. (2017). Sslongi (*S. s. longirostris*) and SSgallaica (*S. s. gallainca*) represent haplotypes of outgroup evolutionary lineages published by Vences et al. (2014).

Table S1. Tissue sampling sites and isolates identification.

Table S2. Phenotype sampling sites and colour pattern identification (1 – striped, 2 – intermediate, 3 – spotted).

(https://www.ivb.cz/wp-content/uploads/JVB-vol.-70-2-2021-Brejcha-et-al.-Fig.-S1-Tables-S1-S2-1.pdf)

Příloha č. 3

Odborný životopis a seznam publikací autora

Curriculum vitae

Mgr. Ing. Jan Chmelař

Education:

since 2014: doctoral studies in Zoology, Faculty of Science, Charles University

2019 – 2022: Master's degree in Environmental protection, specialisation Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences
(Master thesis: "Monitoring of the pathogen *Batrachochytrium salamandrivoran*s within populations of fire salamander *Salamandra salamandra* in Středočeský region")

2011 - 2014: Master's degree in Zoology, specialisation Ecology and Ethology, Faculty of Science, Charles University
(Master thesis: "Ecology, ethology and variability of the european green lizard *Lacerta viridis* in Natural reservation Tiché údolí")

2007 - 2011: Bachelor's degree in Biology, Faculty of Science, Charles University
(Bachelor thesis: "Territoriality in lizards")

Work experience:

2010 – 2022: CTB, a.s. - project manager, geotechnical supervision and support

since 2022: Czech Environmental Inspectorate – department of International Biodiversity Protection and CITES - inspector

Teaching activities:

Ethological methods (practical course)
Ethology and sociobiology (practical course in Prague Zoo)
Sociobiology and behavioral ekology (practical course in Dvůr Králové Zoo)
Field course in zoology (practical course)
Vertebrate Zoology (practical course)
Morphology of animals (practical course)

Active participation at conferences:

2017 - Zoo je věda (Zoo is a science, conference of the Prague Zoo) – presentation

2017 - Herpetological seminar of the Nature Conservation Agency of the Czech Republic – presentation

2018 – 33rd conference of the Czech Herpetological Society - presentation

2019 – 34th conference of the Czech Herpetological Society - poster

Membership:
Czech Herpetological Society

Deutsche Gesellschaft für Herpetologie und Terrarienkunde (German Society for Herpetology and Herpetoculture)

IMPEL (The European Union Network for the Implementation and Enforcement of Environmental Law)

WTEG – EU Wildlife Trade Enforcement Group

Publications

With IF:

Brejcha, J., Benda, P., Jablonski, D., Holer, T., **Chmelař, J**., & Moravec, J. (2021). Variability of colour pattern and genetic diversity of *Salamandra salamandra* (Caudata: Salamandridae) in the Czech Republic. *Journal of Vertebrate Biology*, *70*(2), 21016-1.

Chmelař, J., Civiš, P., Fischer, D., Frynta, D., Jeřábková., L, Rudolfová, V. & Rehák. I. (2023). Protecting isolated reptile populations outside their main area of distribution: a predictive model of the Dice snake, *Natrix tessellata*, distribution in the Czech Republic. *Biodiversity Data Journal*, 11**.**

Chmelař, J., Frynta, D., Rudolfová, V., Fischer, D. & Rehák. I. (2024). Analysis of microhabitat preferences in the European green lizard (*Lacera viridis*) - a tool for conservation management of isolated populations. *Herpetozoa*. Date submitted: 12 February 2024

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Chmelař, J., Civiš, P., Fischer, D., Frynta, D., Jeřábková., L, Rudolfová, V. & Rehák. I. (2024). One snake to eat them all: A predictive model of *Coronella austriaca* distribution in the Czech Republic and its comparison to syntopic reptile species. *Amphibia-Reptilia*. Date submitted: 24 February 2024

Without IF:

Fischer, D., Velenský P., **Chmelař, J.** & Rehák, I. (2016). European green lizard (*Lacerta viridis*) at the territory of Prague zoo. *Gazella* 43: 37-59.

Chmelař, J., Civiš, P., Fischer, D., Frynta, D., Jeřábková. L. & Rehák, I. (2020). Distribution of the European green lizard, *Lacerta viridis* (Squamata: Lacertidae), in the Czech Republic: Real data and a predictive model. *Acta Societatis Zoologicae Bohemicae*, 84, 1-12.