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Morfologická variabilita vybraných evropských druhů podčeledi Soricinae

Morphological variation of selected European Soricinae shrews

Disertační práce

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Prohlášení

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V Praze, dne 31. 10. 2019   Mgr. Lucie Nováková

Neomys milleri (autor: J. Hošek)

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Abstrakt

Rejskové podčeledi Soricinae tvoří jedny z nejpočetnějších a nejběžnějších zástupců savčí fauny Holarktické oblasti. Jejich uniformní fenotyp a skrytý způsob života však znesnadňuje jejich výzkum. V důsledku toho zákonitosti, které ovlivňují jejich fenotyp a geografickou variabilitu, zůstávají z velké míry neobjasněny. V této disertační práci jsem se proto zaměřila na vybrané aspekty morfologické variability tří zástupců této podčeledi. V první části disertační práce jsme otestovali vliv pohlaví a věku na morfologickou variabilitu krania rejska obecného (*Sorex araneus*) v rámci jedné populace. Odhalili jsme sexuální dimorfismus u rejsků a také rozdíly mezi věkovými skupinami. Na základě této studie byly vybrány vhodné kraniální proměnné pro navazující studie. V druhé části projektu jsme se zaměřili na geografickou variabilitu rejska obecného (*Sorex araneus*) v Evropě v prostoru mezi Baltickým a Jaderským mořem. Zjistili jsme vliv zeměpisné šířky i délky a také nadmořské výšky na velikost rejsků. Ta také výrazně korelovala s vybranými klimatickými proměnnými. Třetí část disertačního projektu byla věnována popisu Dehnelova fenoménu a zjištění případného geografického trendu v jeho průběhu. Zjistili jsme, že zmenšení a následné opětovné dorůstání mozkovny je korelováno s rozdílnými klimatickými faktory pro obě uvedené fáze. Získané výsledky naznačují odlišné evoluční procesy a tlaky, které ovlivňují změnšování a dorůstání mozkovny. Intenzita Dehnelova fenoménu se zvyšuje ve směru na severovýchod. V této práci také prezentujeme dosud nepublikovaná data o průběhu Dehnelova fenoménu ve čtyřech evropských populacích. Poslední část disertačního projektu byla zaměřena na rozlišení dvou druhů z větší části sympatrických rejsců rodu *Neomys*. Popsali jsme rozdíly ve velikosti a tvaru mandibuly mezi populacemi rejsce vodního (*Neomys fodiens*) a rejsce černého (*Neomys milleri*) z oblasti východního Německa a jihu Balkánského poloostrova. Zatímco rejsec vodní na tomto území nevykazoval téměř žádné změny ve vybraných morfologických znacích, rejsec černý se zmenšoval směrem na severozápad. V této práci také popisujeme morfologické rozdíly mezi sympatrickými a alopatrickými populacemi rejsců. Předložená disertační práce si klade za cíl přispět k poznání některých zákonitostí morfologické variability u vybraných Evropských zástupců podčeledi Soricinae a mohl by sloužit jako základ pro plánované navazující studie.

Klíčová slova rejskovití; *Sorex*; *Neomys*; geometrická morfometrie.

Abstract

Soricinae shrews are one of the most common and abundant mammalian group in Holarctic Region. The uniform phenotype and hidden way of life make the research on this group challenging. Basic rules that drive their phenotype and morphological variation still remain unclear. The aim of this dissertation is to clarify the selected aspects of morphological variation in three European Soricinae species. The first part of this thesis is aimed on age and sex skull variation in a single population of the common shrew (*Sorex araneus*). We found significant sexual dimorphism and differences between age groups. Based on this study we selected cranial variables suitable for following studies. The second part of the dissertation project was aimed on geographic variation of the common shrew in the area between the Baltic and the Adriatic sea. We found correlation between size of shrews and latitude, longitude as well as altitude. Size was also correlated with selected climatic variables. The third part of the project was focused on a description of Dehnel´s phenomenon and its geographic pattern. We found that decrease and regrowth of the braincase is separately correlated with different climatic variables. Our results suggest that different evolutionary processes and pressures are involved with two phases of Dehnel´s phenomenon. The intensity of Dehnel´s phenomenon increase towards the north-east. We also presented new data about Dehnel´s phenomenon in four european populations. The last part of the thesis is aimed on discrimination of two partly sympatric water shrews of genus *Neomys*. We described size and shape differences in mandible of *Neomys fodiens* and *Neomys milleri* from two areas – eastern Germany and southern part of the Balkan Peninsula. While *Neomys fodiens* did not show any difference between the two areas, *Neomys milleri* is getting smaller towards the north-west. In this study we also described differences between sympatric and allopatric populations of *Neomys* species. The aim of the presented thesis is to contribute to the knowledge of morphological variation in Soricinae shrews and to serve as a foundation for the future studies.

Keywords red-toothed shrews; *Sorex*; *Neomys*; geometric morphometrics.

Obsah

Seznam předložených prací:

I.   **Nováková Lucie** & Vohralík Vladimír (2017): Age and sex skull variation in a model population of the common shrew (*Sorex araneus*). Folia Zoologica, 66: 254 − 262.

II. **Nováková Lucie**, Lázaro Javier, Kryštufek Boris & Vohralík Vladimír: Converse Bergmann´s rule drives the size pattern in the common shrew (*Sorex araneus*) in Europe. Manuskript.

III. Lázaro Javier, **Nováková Lucie**, Hertel Moritz, Taylor Jan R. E., Muturi Marion, Zub Karol & Dechmann Dina K. N.: Seasonal reversible size changes in small high-metabolic mammals exceed geographic variation - a review of Dehnel's Phenomenon in the common shrew. Manuskript.

IV. **Nováková Lucie** & Vladimír Vohralík (2019): Discrimination of the sympatric species of water shrews *Neomys fodiens* and *N. milleri* (Soricomorpha, Soricidae). Zoologischer Anzeiger, 283: 27 – 32.

Úvod

Rejskové, rejsci a bělozubky (Soricidae) s více než čtyřmi sty druhy tvoří jednu z druhově nejpočetnějších čeledí savců (Hutterer 2005). Tato skupina díky svému starobylému vzhledu a spoustou unikátních apomorfií přitahuje pozornost badatelů již od 19. století (např. Fischer 1813 – 1814, Rope 1873). Jedna z podčeledí, Soricinae, tedy „červenozubí rejskové", jsou jedni z nejpočetnějších a nejrozšířenějších savců Holarktické oblasti. Jejich skrytý způsob života a velmi uniformní fenotyp napříč celou skupinou ovšem velmi znesnadňuje jejich výzkum. Dokonce i v tak probádaném území jako je Evropa stále dochází k rozlišení (staro)nových druhů, např. *Sorex antinorii* a *S.arunchi* (Hutterer 2005, Lapini & Testone 1998) nebo *Neomys milleri* (Igea et al. 2015). Důvody proč jsou tak evolučně úspěšní a jakými pravidly se řídí jejich morfologická variabilita napříč geografickými škálami zůstávají stále neobjasněny. Popis a detailní studium rejsčí morfologie tedy stále může nabídnout nové a netušené objevy.

Jako modelový druh pro studium rejskovitých hmyzožravců byl vybrán rejsek obecný *Sorex araneus* Linnaeus, 1758. Rejsek obecný je nejběžnější evropský hmyzožravec, který je studován již několik staletí (např. Rope 1873) a s velkou intenzitou včetně odchytů a tvorby rozsáhlých sbírek již od počátku minulého století (Miller 1912, Ognev 1928). Z tohoto důvodu je jedním z nejhojněji zastoupených drobných savců v evropských sbírkách, včetně sérií zohledňující různá kritéria odchytu (sběry z průběhu celého kalendářního roku, série z jedné lokality zahrnující několik desítek let atp.). Rejsek obecný je tedy ideálním druhem pro studium morfologické variability podčeledi Soricinae. Dalšími zástupci vybranými pro studium morfologické variability jsou dva druhy rejsců rodu *Neomys*, konkrétně *Neomys fodiens* (Pennant, 1771) a *Neomys milleri* Mottaz, 1907. *Neomys fodiens* a *N. milleri* jsou v části jejich areálů sympatričtí a jsou výrazně přizpůsobeni vodnímu prostředí (Hutterer 1985). I když se míra jejich přizpůsobení liší (*N. fodiens* je více akvatilní), v jihovýchodní části překryvu jejich areálů je velmi obtížné je rozlišit.

Pro studium morfologické variability zmíněných druhů byly použity metody jak klasické tak geometrické morfometrie. Měření lineráních rozměrů je jedním ze standardních přístupů v mammalogii a existuje tedy celá řada srovnatelných literárních údajů. V případě nedostatečnosti lineárních rozměrů pro zodpovězení námi řešených problémů jsme použili metody geometrické morfometrie, která umožňuje vyhodnotit nejen metrické, ale i tvarové rozdíly.

Cíle práce

V kontextu současných znalostí o morfologické variabilitě a biologii zástupců podčeledi Soricinae byly formulovány čtyři cíle práce:

1) Detailně popsat vnitropopulační variabilitu kraniálního skeletu nejběžnějšího zástupce evropských rejskovitých, rejska obecného (*Sorex araneus*) a otestovat vliv věku a pohlaví na jeho kraniální proměnné. (Spis I)

2) Otestovat vliv geografické polohy a environmentálních faktorů na vybrané kraniální proměnné u vybraných evropských populací rejska obecného (*Sorex araneus*). (Spis II)

3) Popsat průběh a geografickou variabilitu Dehnelova fenoménu u rejska obecného (*Sorex araneus*). (Spis III)

4) Rozlišit dva druhy sympatrických rejsců rodu *Neomys* v různých částech jejich areálu výskytu s ohledem na jejich sympatrický či alopatrický výskyt. (Spis IV)

Morfologická variabilita vybraných zástupců podčeledi Soricinae

Na základě prací zahrnutých v předložené disertační práci se v této části budu zabývat vybranými aspekty morfologické variability rejska obecného a rejsců vodního a černého.

Vnitropopulační variabilita

Nezbytným předpokladem studia variability jakéhokoliv druhu živočicha (např. geografické variability) je detailní analýza jeho variability na populační úrovni, tj. otestovat vliv pohlaví (sexuální dimorfismus), změny ke kterým dochází během růstu a stárnutí, popřípadě prozkoumat další fenomény, které se na populační úrovni mohou vyskytovat a vytvářet rozdíly mezi jedinci.

Pro rozbor vnitropopulační variability jsme zvolili rejska obecného (*Sorex araneus*). Rejsek obecný se ve volné přírodě dožívá cca 15 měsíců. Mláďata se rodí v období duben až říjen. Ti, kteří přežijí zimu, na jaře pohlavně dospějí, následně se rozmnoží a zemřou před druhou zimou svého života (Pucek 1970). Velmi zřídka se vyskytují výjimky, kdy se tohoroční samice rozmnožují již před svojí první zimou (Vlasák 1997). Z toho důvodu se v přírodě vyskytují najednou nanejvýš dvě věkové kohorty rejsků (tohoroční a jednou přezimovavší), vyjma zimy, kdy se vyskytuje pouze jediná věková kohorta (rejsci narození během předchozí reprodukční sezony).

Kromě studií věnovaných Dehnelovu fenoménu (viz dále), literatury obsahující rozbor věkové variability kraniálních rozměrů u rejsků je poskrovnu. Většina studií se zabývá rozdíly v kondylobazální délce nebo výšce neurokrania (např. Kubik 1951; Pucek 1955, 1963). Detailnější rozbor na rozsáhlejším materiálu byl proveden teprve Homolkou (1980) a Spitzenbergerovou & Bauerem (2001). V naší studii (Nováková & Vohralík 2017) jsme rozdělili rejsky do dvou věkových skupin, juvenilové (tohoroční jedinci) odchycení v období září až listopad a adulti (přezimovavší jedinci) odchycení v období duben až listopad. Ze 14 měřených rozměrů na mandibule a kraniu jsme zjistili rozdíly u sedmi z nich – vyšší hodnoty u adultů byly u výšky a délky mandibuly, v tloušťce mandibuly měřené pod prvním molárem, u kondylobazální délky a také u délky a šířky neurokrania. U adultů byl zjištěn kratší první dolní řezák než u juvenilních jedinců (zřejmě způsobeno obrusem). Naše výsledky jsou v souladu s již publikovanými poznatky, výjimku tvoří autoři, kteří nenašli žádný rozdíl v kondylobazální

délce mezi věkovými skupinami (např. Dehnel 1949, Schubarth 1958, Spitzenberger & Bauer 2001).

Sexuální dimorfismus je u rejsků obecně považován za zanedbatelný (Churchfield 1990). Navzdory tomu, že u postkraniálního skeletu byl výrazný dimorfismus u rejska obecného prokázán (Dolgov 1961, 1985; Brown & Twigg 1970), u kraniálního skeletu většina autorů vliv pohlaví nijak netestovala nebo nalezla pouze zanedbatelné rozdíly (Dehnel 1949, Homolka 1980, Hůrka 1986, Hausser et al. 1990, Mishta 2007, Churchfield & Searle 2008). Naopak v naší studii jsme prokázali výrazné rozdíly u krania ve třech studovaných rozměrech – tloušťka mandibuly měřená pod prvním molárem, délka prvního dolního řezáku a šířka neurokrania. Větší rozdíly byly popsány u adultních jedinců, kde samci měli robustnější mandibulu, delší dolní řezák a širší neurokranium (Nováková & Vohralík 2017). Zvláštní zřetel zaslouží šířka neurokrania, která u adultních samic byla prakticky stejná jako u juvenilů obou pohlaví, zatímco samci měli neurokranium výrazně širší. To naznačuje možné rozdíly v průběhu Dehnelova fenoménu (viz podkapitola Dehnelův fenomén) u samců a samic.

Obecně lze říct, že největší změny během postnidálního života rejska probíhají na neurokraniu. Proto při porovnávání různých populací je vhodné použít rozměry na mandibule (bez zahrnutí prvního řezáku) a/nebo rostru. I když v literatuře bývá často zanedbáván, sexuální dimorfismus u rejsků existuje. Rozdíly mezi pohlavím se více projevují na adultních jedincích, proto při využití materiálu bez rozlišení pohlaví je vhodné použít juvenilní (tj. nepřezimovavší) jedince. Na výsledky předloženého rozboru kraniální variability v rámci jedné populace lze navázat studiemi zaměřenými na další faktory ovlivňující morfologii kraniálního skeletu u rejska obecného.

Geografický pattern a vliv environmentálních faktorů

I přes množství studií věnovaných morfologii rejska obecného (*Sorex araneus*), zůstávají obecná pravidla, která formují jeho fenotypovou variabilitu, stále neznámá. Ani četné studie navazující na výzkum chromosomových ras neodhalily žádnou jednoznačnou souvislost mezi chromosomovou rasou a morfologií kraniálního skeletu (recentně shrnuli Polly & Wójcik 2019). Jedním z nejvíce citovaných zoogeografických pravidel vysvětlujících morfologickou variabilitu ptáků a savců na větších geografických škálách je Bergmannovo pravidlo. Výjimku z tohoto pravidla tvoří někteří drobní masožraví savci, např. rejsci rodu *Sorex* (Pucek 1970, Schipanov et al. 2011) a lasice kolčava (Abramov & Baryshnikov 2000, LaPoint et al. 2017).

Eurasijští rejsci rodu *Sorex* dokonce následují pravidlo zcela opačné (Ochocińska a Taylor 2003). Abychom objasnili, které faktory určují morfologickou variabilitu u rodu *Sorex*, zvolili jsme nejběžnějšího a nejpočetnějšího zástupce rejskovitých v Evropě – rejska obecného (*Sorex araneus*), u kterého jsme testovali vliv zeměpisné délky a šířky, nadmořské výšky a řady klimatických faktorů na morfologii jeho mandibuly (Nováková et al. - přiložená práce II).

Studovali jsme materiál mandibul 1155 jedinců rejska obecného pocházející z 29 populací obývajících geograficky velmi členitý prostor, který se rozkládá od Baltického moře na severu po Jaderské moře na jihu. Prokázali jsme mezipopulační variabilitu jak ve velikosti, tak i v tvaru mandibuly. Na studovaném materiálu jsme prokázali zmenšování rejsků směrem na sever, což je ve zřejmém rozporu s Bergmannovým pravidlem, ale zároveň zvětšování ve směru od západu na východ. Značný vliv má také nadmořská výška, která pozitivně koreluje s velikostí zkoumaných rejsků. Jedním z nejdůležitějších faktorů ovlivňujících velikost madibuly a tedy i celkové velikosti rejsků se ukázala precipitace, jejíž vysoká hodnota bývá spojována s hojnějším výskytem nejběžnější složky potravy rejsků - žížal (Churchfield 2002). Největší a tvarově nejodlišnější mandibuly vykazovali rejsci ze slovinské hory Snežnik, která představuje v našem materiálu lokalitu s nejvyšším ročním úhrnem srážek. Proto, ačkoliv naše výsledky založené na analýze velikosti rejsků a zeměpisné šířky potvrdily obrácený trend než definuje Bergmannovo pravidlo (Ochocińska & Taylor 2003), některé otázky zůstávají stále otevřené. Zajímavým tématem dalšího výzkumu proto bude objasnění vzájemného vztahu velikosti jedinců, nadmořské výšky a precipitace. To může být nejlépe provedeno analýzou materiálu rejsků získaných v různých nadmořských výškách v rámci jednoho horského masivu.

Dehnelův fenomén

Zmenšování lebky, mozku a některých vnitřních orgánů před obdobím zimy a jejich opětovné zvětšování následující jaro se označuje jako tzv. Dehnelův fenomén. Poprvé byl popsán polským badatelem Augustem Dehnelem (Dehnel 1949) při studiu lebky rejska obecného (*Sorex araneus*). Donedávna byl Dehnelův fenomén předmětem kontroverzí, mnozí autoři zpochybňovali jeho existenci a zjištěné rozdíly přisuzovali vymírání kohort jako je tomu například u severoamerického rodu *Blarina* (Dapson 1968). I když existují náznaky, že Dehnelův fenomén by nemusel být přítomen pouze u rejskovitých, ale obecně u menších savců s velmi rychlým metabolismem (Dechmann et al. 2017), nezvratně byl potvrzen pouze u rejska obecného *Sorex araneus* (Lázaro et al. 2017).

Také jediná review shrnující poznatky o Dehnelově fenoménu je založena převážně na materiálu a publikacích o rejskovi obecném (Pucek 1970, Obr. 1). Pucek (1970) popsal geografický trend Dehnelova fenoménu v Evropě a dospěl k závěru, že k intenzivnějšímu zmenšování (výška mozkovny) dochází u populací na severu a východě kontinentu. U východněji žijících populací dochází také k rychlejšímu dorůstání po zimě v porovnání se západními populacemi.
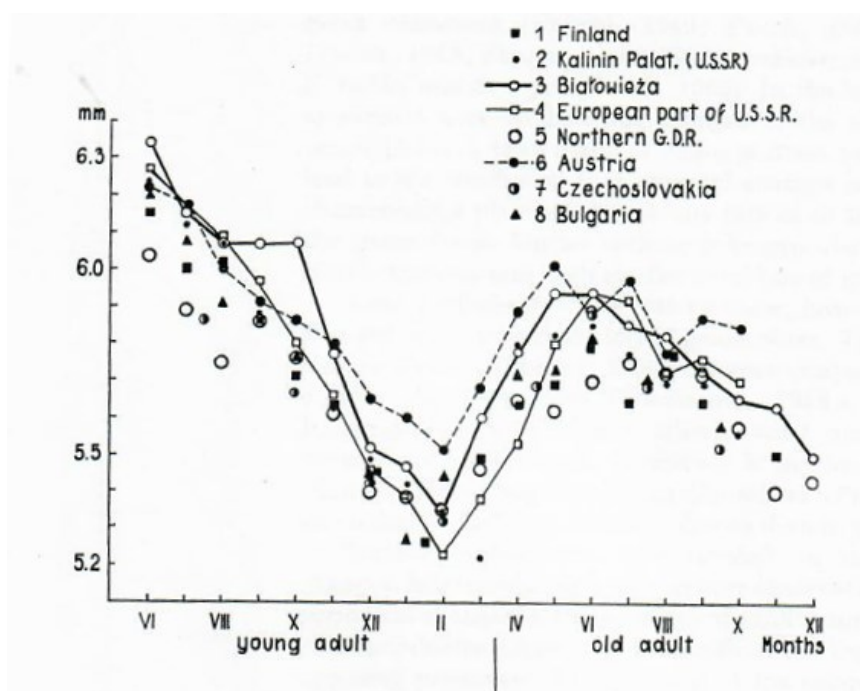

Fig. 1. Changes in height of the brain-case in *Sorex araneus* from some of the populations studied. 1. Finland (data from Skarén, 1964), 2. Kalinin Palatinate, USSR (unpublished data of L. V. Viktorov), 3. Białowieża Primeval Forest, Poland (data from Pucek, 1955), 4. European part of USSR (data from Pucek, 1963), 5. Northern part of German Democratic Republic (data from Schubarth, 1958), 6. Austria (data from Spitzenberger, 1964), 7. Czechoslovakia, 8. Bulgaria (data from Pucek and Markov, 1964).

Obr. 1 Změny ve výšce neurokrania v osmi evropských populacích rejska obecného (*Sorex araneus*). Převzato z Pucek (1970).

V přiloženém manuskriptu (Lázaro, Nováková et al. - přiložená práce III) jsme zkompilovali dostupnou literaturu zabývající se Dehnelovým fenoménem u rejska obecného, získali jsme data o čtyřech dalších dosud nepublikovaných populacích rejska obecného a také jsme se zabývali Dehnelovým fenoménem u dalších druhů drobných savců. Testovali jsme vliv geografických a klimatických proměnných na změny velikosti mozkovny, změny různých oblastí mozku a tělesné váhy. Změny mozkovny jsme rozdělili do dvou fází – podzimní/zimní

depresi a jarní nárůst. Průměrná zimní změna výšky neurokrania představovala její depresi o 13 % a jarní nárůst o 10 %. Deprese a nárůst výšky mozkovny korelovaly s rozdílnými klimatickými proměnnými. Je tedy možné a pravděpodobné, že podzimní/zimní deprese a jarní nárůst jsou dva nezávislé procesy, které jsou regulovány každý jinými faktory. Porovnávali jsme nově získaná data o sezónních změnách v různých částech mozku s již publikovanými daty z Ruska (Yaskin 1984, 1994). Nenalezli jsme žádný zřejmý trend avšak pro nedostatek dat z dalších oblastí areálu výskytu rejska, nebylo možné vyvodit žádné konkrétní závěry. Zkompilovaná data o tělesné hmotnosti rejsků vykazovala výrazné výkyvy váhy v průběhu roku (zimní úbytek o 21 % a následný nárůst až o 82 %). Hmotnost jedince však není vhodnou proměnnou pro demonstraci Dehnelova fenoménu, protože kolísání váhy je způsobeno také dalšími faktory (například v důsledku zimního nedostatku potravy) a nejen změnou velikosti mozkovny, mozku a vnitřních orgánů. Proto poukazujeme na fakt, že váha by v tomto případě měla být uváděna pouze jako jeden z parametrů spolu s dalšími proměnnými.

Rozlišení dvou sympatrických druhů rejsců rodu *Neomys*

V rámci rodu *Neomys* jsou v současnosti rozlišovány čtyři recentní druhy (Igea et al. 2015, Querejeta & Castresana 2018), z toho dva (*N. fodiens* a *N. milleri*) s poměrně velkým areálem rozšíření pokrývajícím značnou část evropského kontinentu. Jejich rozlišením na základě kraniálních rozměrů se zabývalo množství autorů s různými výsledky (např. Spitzenberger 1980, Kryštufek & Griffiths 2000, Popov & Zidarova 2008). Obecně lze oba tyto druhy v oblasti Německa, ČR a Polska rozlišit poměrně dobře podle velikosti (Rempe & Bühler 1969, Ruprecht 1971), zatímco směrem dále na jihovýchod je rozlišení stále obtížnější až k extrému na jihu Balkánského poloostrova, kde rozlišení druhů je možné pouze na základě kombinace několika tělesných a kraniálních rozměrů (Vohralík & Sofianidou 1987, Spitzenberger 1990), detailní morfologické analýzy samčích pohlavních orgánů (Pucek 1964) či pomocí molekulárních metod.

My jsme porovnávali populace obou druhů rejsců z jihu Balkánského poloostrova (Bulharsko, Řecko) a z východní části Německa (Nováková & Vohralík 2019). Jedná se o oblasti, kde končil areál výskytu vždy jednoho ze studovaných druhů, a proto bylo možné porovnávat jak populace žijící alopatricky, tak i populace obou druhů žijící v sympatrii. Zjistili jsme, že v souladu s literaturou, německé populace obou druhů lze od sebe snadno rozlišit i pomocí lineárních rozměrů mandibuly, zatímco situace u balkánských populací je

komplikovanější. Zatímco mandibula druhu *Neomys fodiens* byla v obou zkoumaných oblastech prakticky identická, druh *Neomys milleri* z německých populací byl znatelně menší než *N. milleri* z Balkánu a než *N. fodiens* z obou oblastí. Při porovnání mandibul sympatrických a alopatrických *N. fodiens* z Německa se ukázalo, že sympatrický *N. fodiens* je tvarově podobnější německému *N. milleri* než v alopatrii žijící *N. fodiens*. Obdobná situace je na Balkáně při porovnání sympatrických a alopatrických *N. milleri*, kde sympatrické populace *N. milleri* jsou podobnější balkánskému *N. fodiens* (který zde žije pouze v sympatrii s *N. milleri*) než v alopatrii žijící populace *N. milleri*. Co se týče velikosti mandibuly, v sympatrii s jiným druhem žijící populace jsou v obou oblastech menší než populace alopatrické.

Závěry

V předložené disertační práci jsem se zabývala různými aspekty morfologické variability rejska obecného (*Sorex araneus*), rejsce vodního (*Neomys fodiens*) a rejsce černého (*Neomys milleri*). U rejska obecného byl u některých lebečních rozměrů zjištěn pohlavní dimorfismus a věková variabilita. Největší vliv pohlaví a věku byl zjištěn u neurokrania. Z toho důvodu není vhodné používat neurokranium pro komparativní studie různých populací rejska, vhodnější je využít některé rozměry a především tvar mandibuly či rostra. U rejska obecného byl v kontextu střední Evropy a přilehlých oblastí zjištěn geografický gradient. Se zvětšující se zeměpisnou šířkou se velikost rejska zmenšuje, naopak se zvětšující se zeměpisnou délkou a nadmořskou výškou se mandibula rejska (a tedy i celý jedinec) zvětšuje. Tvarově nejodlišnější mandibuly, v porovnání s ostatními zkoumanými populacemi, byly zjištěny u populace ze slovinské hory Snežnik. Tuto tvarovou odlišnost lze vysvětlit pomocí alometrie, otázkou ovšem zůstává, proč jsou rejsci v dané oblasti největší ze všech 29 námi studovaných populací. Ze získaných dat neplynou závěry, které by umožnily jednoznačně formulovat nějaké nové morfologicko–geografické pravidlo, ale přinášíme nové poznatky o vlivu geografických i klimatických proměnných na rejska obecného.

Dále jsem se zabývala Dehnelovým fenoménem, jeho průběhem a možným geografickým patternem. Potvrdili jsme gradient ve změně velikosti mozkovny, který ukazuje výraznější prohlubování Dehnelova fenoménu směrem na severo-východ a tedy možnost, že se jedná o adaptaci na prostředí. Ukázali jsme, že podzimní/zimní deprese a opětovný jarní nárůst mozkovny jsou separátně korelovány s různými klimatickými faktory a tedy zřejmě modulovány rozdílnými evolučními procesy a tlaky. Prezentovali jsme nové poznatky o

remodelaci mozku. V manuskriptu také shrnujeme dosavadní publikovanou literaturu týkající se Dehnelova fenoménu nejen u rejska obecného, ale také u dalších drobných savců.

V poslední předložené práci jsme se zabývali rozdíly ve velikosti a tvaru mandibuly u dvou druhů rejsců rodu *Neomys* na okrajích jejich areálu výskytu ve střední Evropě a na Balkánském poloostrově. Zatímco *Neomys milleri* se v rámci těchto oblastí značně (hlavně ve velikosti) mění, tj. zmenšuje se směrem na severo-západ, *Neomys fodiens* zůstává ve zkoumaném geografickém prostoru téměř stejný. To způsobuje, že v některých částech překryvu svých areálů jsou oba druhy rejsců snadněji rozlišitelné na základě své velikosti. Naopak v jiných oblastech způsobuje jejich rozlišení problémy. V případě sympatrického výskytu rejsců, jsou si jejich mandibuly tvarově podobnější a oba druhy jsou menší než při porovnání s alopatrickými populacemi. Z těchto výsledků vyplývá, že rejsci se výrazně přizpůsobují danému prostředí, vliv kompetice se omezuje pouze na změnšení jejich velikosti.

Z předložených prací vyplývá, že u zkoumaných zástupců podčeledi Soricinae je zřejmě mimořádně důležitým evolučním faktorem dostupnost potravy. Nejvýraznější rozdíly mezi populacemi rejska obecného z různých částí Evropy byly ve velikosti jedinců, která pozitivně korelovala s precipitací na dané lokalitě. Množství precipitace je spojováno s dostupností hlavní složky potravy rejska obecného, tj. žížal. Obdobnou pozitivní korelaci jsme zjistili mezi precipitací a intenzitou opětovného zvětšování mozkovny rejska obecného po zimním období. Také tvarová i velikostní podobnost mandibul obou zkoumaných druhů rejsců rodu *Neomys,* která je větší při jejich výskytu v sympatrii než v alopatrii, naznačuje větší důležitost potravy v porovnání s jinými biotickými faktory, včetně vzájemné kompetice.

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Prohlášení o podílu na publikacích

Prohlašuji, že v případě všech společných publikací zařazených do disertační práce Mgr. Lucie Novákové, byl podíl spoluautorky zcela zásadní ve všech fázích přípravy dotyčných publikací. L. Nováková se u všech prací podílela na konceptu práce, zpracovala veškerý použitý materiál, analyzovala data a sepsala manuskript. U dvou již publikovaných prací je hlavním a zároveň korespondenčním autorem.

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To the members of the dissertation committee –

Hereby I confirm that Lucie Nováková has significantly contributed to the manuscript "Seasonal reversible size changes in small high-metabolic mammals exceed geographic variation - a review of Dehnel's Phenomenon in the common shrew". Specifically, Lucie contributed with conceptual ideas, data analyses, writing and general revision of the text to the extent that properly corresponds with her position as second co-author in the manuscript. The extent of her involvement in the manuscript consequently warrants its inclusion in her dissertation.

Sincerely,

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I. **Nováková Lucie** & Vohralík Vladimír (2017): Age and sex skull variation in a model population of the common shrew (*Sorex araneus*). Folia Zoologica, 66: 254 – 262.

Age and sex skull variation in a model population of the common shrew (*Sorex araneus*)

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Abstract. Sexual and age variation of the common shrew was assessed in 173 individuals captured in the Novohradské hory mountain range in South Bohemia, Czech Republic. Age variation was found in seven of the fourteen measurements examined. In six of them (height of mandible, height of mandible measured below the first molar, length of mandible, cranial width, condylobasal length, length of neurocranium), values in adults were higher than those in juveniles, while the opposite was found for the length of the lower incisor. Evidence of sex differences was found only in three measurements: height of mandible measured below the first molar, cranial width and length of the lower incisor. Our results suggest the need for separating age and sex groups in studies of skull variation in *Sorex* shrews.

Key words: morphometry, age variation, sexual dimorphism, Soricidae

Introduction

There are many studies concerned with morphological variation of the common shrew (*Sorex araneus*). Most of them focused on the Dehnel's phenomenon, i.e. the winter reduction of some internal organs and several body and skull measurements (e.g. Dehnel 1949, Pucek 1955, 1963, 1970), or morphological differences between chromosomal races (e.g. Wójcik et al. 2000, Stefen 2013). Relatively detailed information is available about skull development during nidal life of the common shrew (Vogel 1973). Unfortunately, much less attention has been paid to skull variation in shrews during the spring-autumn period. In general, it was assumed that there are no or only negligible sex differences in skull measurements (e.g. Schubarth 1958, Homolka 1980, Poroshin et al. 2010). As to the differences in skull measurements between young in the calendar year of their birth and overwintered individuals, previous studies dealt mostly with changes in braincase height or condylobasal length (e.g. Kubik 1951, Pucek 1955, 1970), with a few exceptions (Homolka 1980, Spitzenberger & Bauer 2001). In fact, detailed analyses of sex and age differences in skull measurements within a common shrew population, based on a large enough sample, are very scarce. We used classic morphometry (linear measurements), as we wanted to retain information about size variation in the population. In addition, this method gives values that are commonly used in the mammalogical literature. It should be stressed that without such analysis it is impossible to distinguish geographical variation due to environmental variables from intrapopulation variation due to sex and age differences. Therefore, the aim of our study was to fill this gap in the literature and make a detailed comparison of skull measurements in two age groups of shrews with the aim of determining if there is sexual dimorphism, especially in adults, which have been rarely studied to date.

Material and Methods

Material
The material used in this study consisted of skulls of common shrews snap-trapped between 1972 and 1976 in the locality Žofín situated in the Novohradské hory mountain range, South Bohemia, Czech Republic. Shrews were collected mostly along streams flowing through a wet meadow, while a smaller part of the material was collected in a nearby beech-spruce forest, at an elevation of ca 750 m. For further details about the locality, see Vohralík et al. (1972).

All captured animals were processed by standard mammalogical methods, i.e. measured, dissected and conserved in 4% formaldehyde. Later, skulls were

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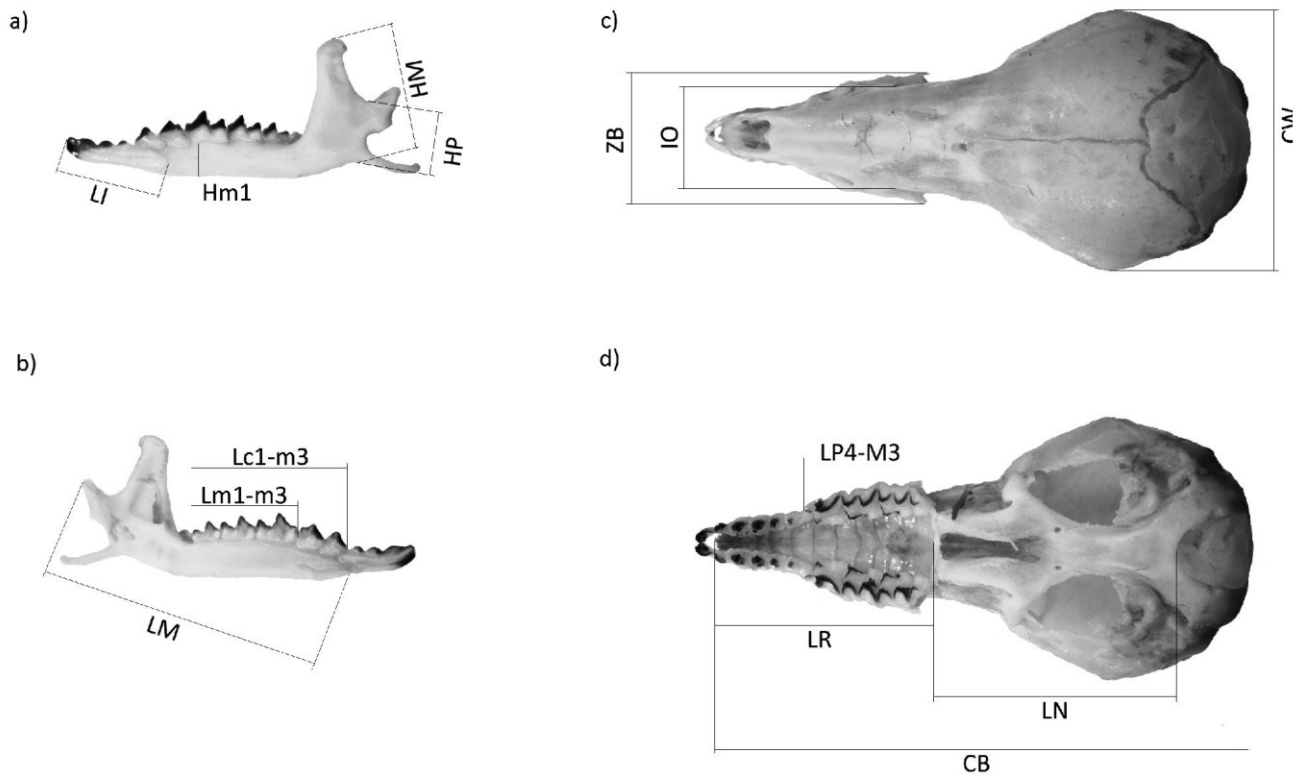
Fig. 1. Mode of taking of the skull measurements. Mandible: a) buccal view, b) lingual view; cranium: c) dorsal view, d) ventral view.
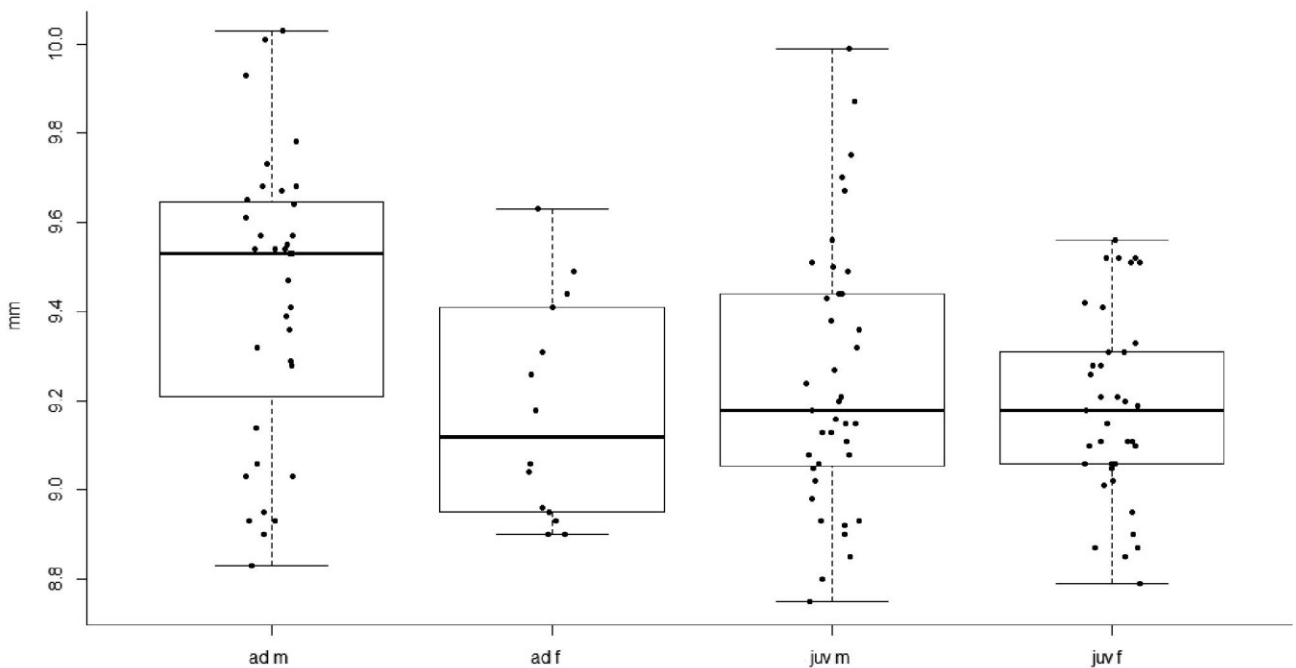
Fig. 2. Variation of the cranial width (CW). Abbreviations: ad m (adult males), ad f (adult females), juv m (juvenile males), juv f (juvenile females). Boxplots show the interquartile range with median for each group. Dots represent individual values, outliers are shown as circles.

extracted and cleaned by *Dermestes maculatus* beetles. We divided the animals (n = 173) into four groups – juvenile males (52 specimens), juvenile females (47 specimens), adult males (53 specimens), and adult females (21 specimens). The juvenile categories include only immature individuals trapped between September and November of the year they were born in. They were identified based on the size of testes in males and absence of embryos and signs of previous parturition in females. In addition, juveniles exhibited

255
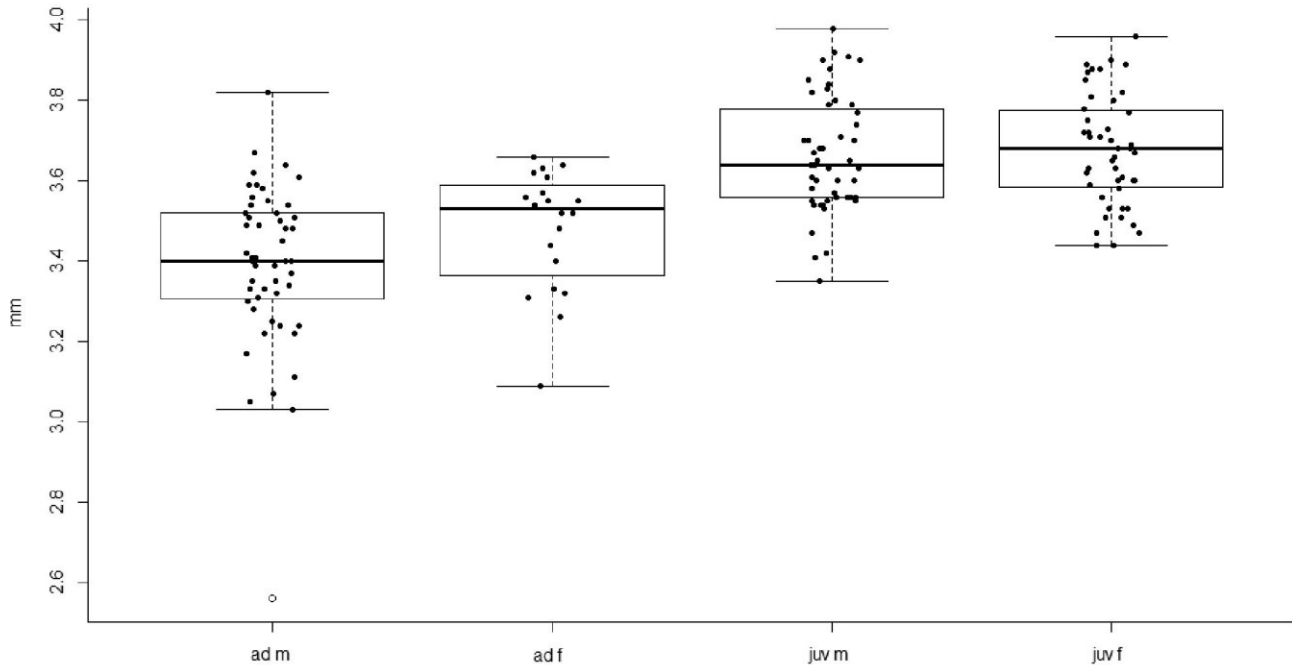
Fig. 3. Variation of the length of the lower first incisor (LI). For description see Fig. 2 legend.
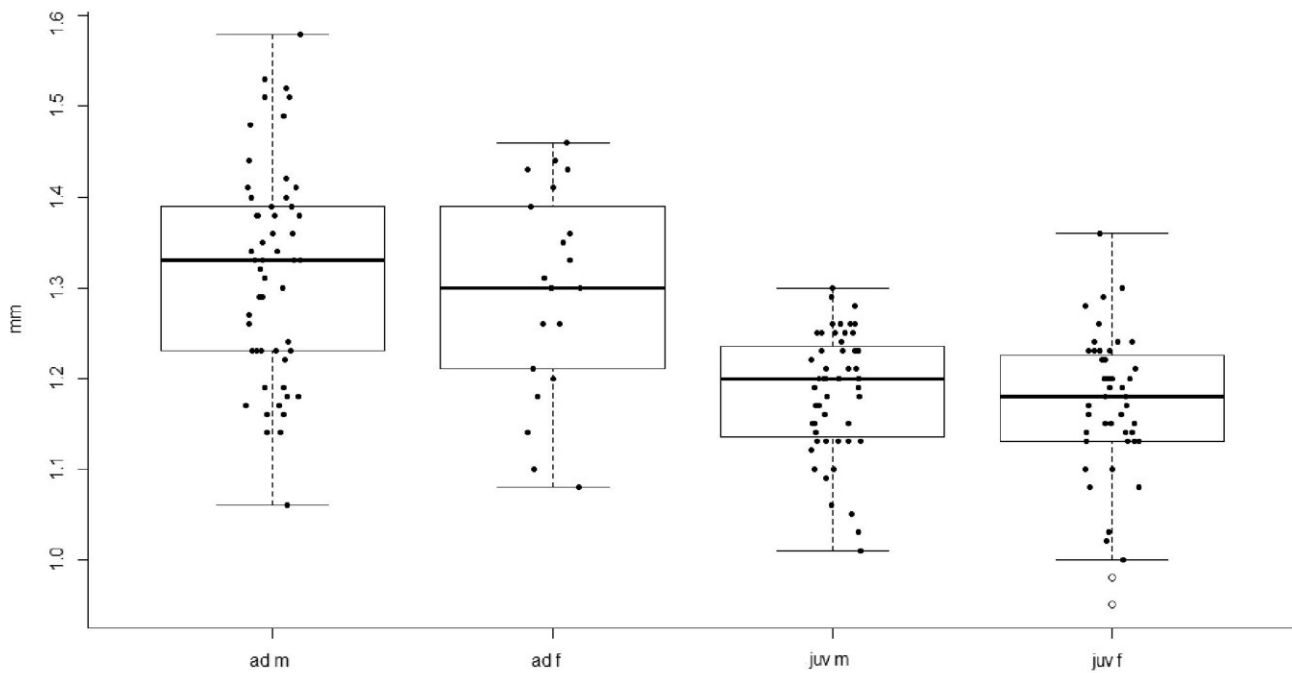
Fig. 4. Variation of the height of mandible measured below the first molar (Hm1). For description see Fig. 2 legend.

visibly more prominent hairs on the tip of their tail and different pelage colour. Teeth of juveniles clearly differ from those of adults by less abrasion. Adults include overwintered individuals trapped between April and November.

Measuring and statistics
Skulls were magnified under an Olympus SZX 12 stereomicroscope and high-resolution photos were taken with an Olympus DP70 camera. Pictures of crania from dorsal and ventral views and left mandibles from buccal and lingual views were taken after placing them on a horizontal surface without any correction of their position. All measurements were recorded from the images in the tpsDig 2 software (Rohlf 2016) to the nearest 0.01 mm. We took seven cranial and seven mandibular measurements mostly according Vesmanis (1976), see Fig. 1. On the buccal

256
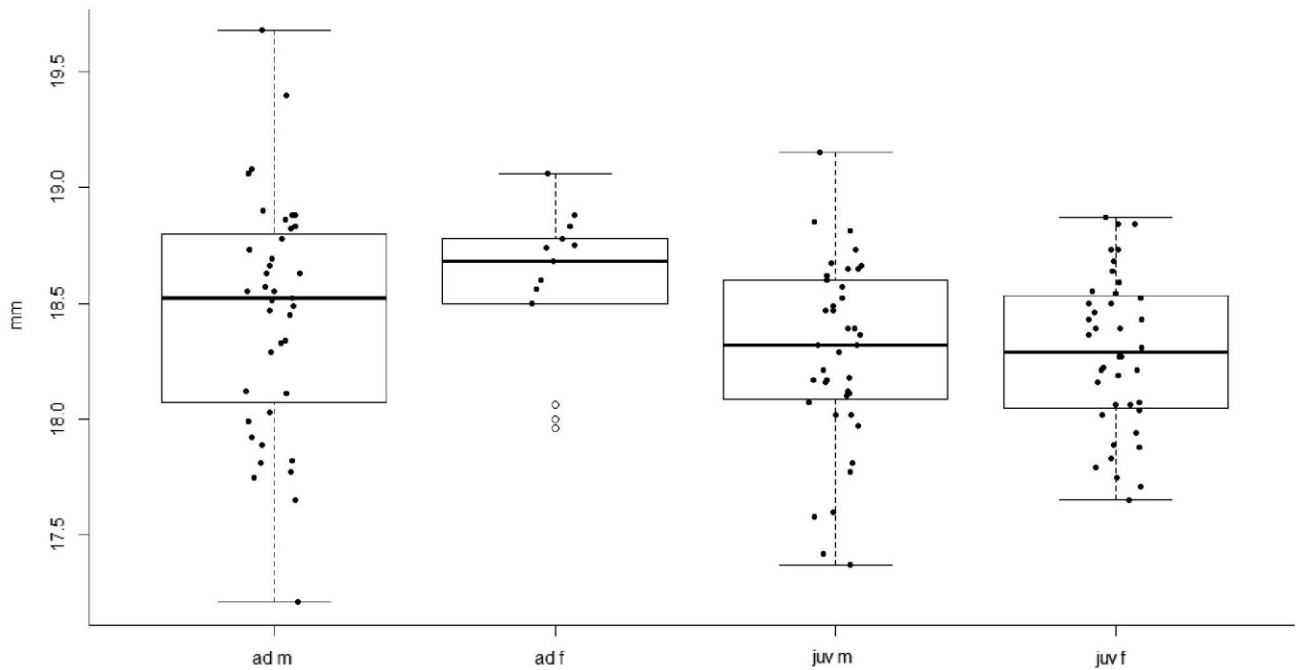
Fig. 5. Variation of the condylobasal length (CB). For description see Fig. 2 legend.

side of the mandible, we measured height of mandible (HM) and postcoronoid height (HP), both taken at the least vertical distance, height of mandible below the first molar (Hm1), measured at the aboral margin of *foramen mentale*, and length of incisor (LI), measured at the greatest length of the visible part of the incisor, without the root. On the lingual side, we measured length of mandible (LM), length of tooth row (Lc1-m3), and length of molar row (Lm1-m3). From the dorsal view of the skull, we measured cranial width (CW), zygomatic width (ZB), and interorbital width (IO). Condylobasal length (CB), length of rostrum (LR), length of neurocranium (LN), and length of upper molariform tooth row (LP4-M3) were measured from the ventral view of the skull. All teeth measurements were taken across crowns, with the exception of LI. One person took all the pictures and performed the measurements.

All variables were normally distributed (Shapiro-Wilk test). The effect of age and sex was tested by two-way ANOVA. Mutual differences between all four groups (juvenile males, juvenile females, adult males, adult females) were tested by independent samples t-test. Using a general linear model (MANOVA), we revealed a significant effect of age and sex on all measurements (age: F = 11.62, $p < 0.001$; sex: F = 2.60, $p = 0.003$). Principal component analysis (PCA) showed correlations between our measurements (see Supplementary material, Table S1). Additionally, we tested the effect of age and sex on the first four principal

components (two-way ANOVA, all results in Table S2) with eigenvalues higher then 1.0 (Table S3). Statistical significance was evaluated at α level of 0.05.

Descriptive statistics, two-way ANOVA and t-tests were performed in the PAST software (Hammer 2016), general linear models and PCA in Statistica 7 software (StatSoft, Inc. 2004). All plots were generated in R version 3.3.3 (R Core Team 2017).

Results

Age variation

Age variation was discovered in seven of the fourteen measurements examined (Tables 1, 2, Figs. 2-5). In six of them, the values in adults were higher than those in juveniles, mostly in both sexes (HM, Hm1, LM, CB, and LN), but only in males in the case of CW (Fig. 2; males: $t_{74} = 2.52$, $p = 0.014$; females: $t_{50} = 0.15$, $p = 0.878$). Conversely, length of the lower first incisor (LI) was much shorter in adults than in juveniles (Fig. 3; F = 100.03, $p < 0.001$). In seven measurements (HP, Lc1-m3, Lm1-m3, ZB, IO, LR, LP4-M3), we did not find any differences between adults and juveniles. Age has a significant effect on all the tested principal components (PC1: F = 9.07, $p = 0.003$; PC2: F = 27.97, $p < 0.001$; PC3: F = 36.93, $p < 0.001$; PC4: F = 6.84, $p = 0.010$).

Sexual dimorphism

Influence of sex as an important variable was found only in three of the fourteen measurements examined

Table 1. Summary statistics. N (sample size), arithmetic mean (mean), SD (standard deviation), min (minimum value), max (maximum value). All measured values are in millimetres. For measurement abbreviations see Material and Methods.

		adults								
		males					females			
	N	mean	SD	min	max	N	mean	SD	min	max
HM	52	**4.48**	0.15	4.22	4.98	21	**4.49**	0.11	4.30	4.71
HP	53	**2.14**	0.10	1.94	2.40	21	**2.16**	0.10	2.00	2.38
Hm1	53	**1.32**	0.12	1.06	1.58	21	**1.30**	0.11	1.08	1.46
LI	51	**3.39**	0.20	2.56	3.82	20	**3.48**	0.15	3.09	3.66
LM	52	**9.73**	0.27	9.11	10.44	20	**9.76**	0.19	9.45	10.36
Lc1-m3	51	**5.30**	0.15	4.88	5.67	20	**5.33**	0.12	5.03	5.56
Lm1-m3	52	**3.68**	0.11	3.46	3.90	21	**3.71**	0.08	3.52	3.87
CW	36	**9.43**	0.32	8.83	10.03	14	**9.18**	0.25	8.90	9.63
ZB	45	**4.81**	0.23	4.31	5.31	16	**4.82**	0.29	4.31	5.39
IO	48	**3.46**	0.16	3.13	3.74	18	**3.48**	0.17	3.24	3.87
CB	39	**18.45**	0.51	17.21	19.68	13	**18.57**	0.35	17.96	19.06
LR	47	**7.86**	0.27	7.20	8.47	17	**7.90**	0.26	7.43	8.36
LN	41	**8.12**	0.24	7.66	8.69	13	**8.17**	0.22	7.76	8.41
LP4-M3	52	**4.28**	0.14	4.00	4.54	19	**4.33**	0.10	4.15	4.49
		juveniles								
		males					females			
	N	mean	SD	min	max	N	mean	SD	min	max
HM	51	**4.45**	0.14	4.18	4.69	47	**4.42**	0.13	4.19	4.69
HP	51	**2.16**	0.08	1.93	2.33	47	**2.15**	0.10	1.93	2.36
Hm1	52	**1.18**	0.07	1.01	1.30	47	**1.17**	0.08	0.95	1.36
LI	51	**3.67**	0.14	3.35	3.98	47	**3.68**	0.14	3.44	3.96
LM	50	**9.63**	0.24	8.99	10.15	47	**9.64**	0.21	9.23	10.01
Lc1-m3	52	**5.29**	0.11	5.07	5.54	47	**5.32**	0.10	5.08	5.48
Lm1-m3	52	**3.67**	0.08	3.42	3.82	47	**3.68**	0.07	3.51	3.83
CW	39	**9.25**	0.30	8.75	9.99	37	**9.19**	0.21	8.79	9.56
ZB	44	**4.76**	0.20	4.33	5.25	43	**4.75**	0.16	4.36	5.12
IO	45	**3.43**	0.13	3.18	3.74	47	**3.44**	0.10	3.23	3.69
CB	40	**18.29**	0.40	17.37	19.15	40	**18.29**	0.33	17.65	18.87
LR	47	**7.85**	0.22	7.33	8.23	47	**7.87**	0.21	7.31	8.27
LN	40	**7.96**	0.21	7.67	8.42	41	**7.91**	0.24	7.49	8.47
LP4-M3	49	**4.28**	0.11	4.00	4.56	47	**4.30**	0.10	4.07	4.59

(Table 2). The height of mandible measured below the first molar (Hm1) was significantly greater in males than in females (t_{172} = 2.47, p = 0.015); confirmed by two-way ANOVA (F = 8.80, p = 0.004; Fig. 4). The cranial width (CW) in adult males was considerably greater than in adult females (Fig. 2; t_{49} = 2.66, p = 0.011). In juveniles, no sexual dimorphism in CW was found (t_{75} = 1.04, p = 0.303). However, it is apparent that in both age categories males attained much higher maximum values than females. Two-way ANOVA revealed a significant main effect of sex on the length of the lower incisor (LI) (F = 12.09, p < 0.001), LI was also the only measurement showing significant interaction between age and sex (F = 6.21, p = 0.014). Although means and medians were higher in females than in males (Fig. 3, Table 1), t-test was insignificant both when comparing adult (t_{70} = 1.77, p = 0.081) and juvenile shrews (t_{98} = 0.48, p = 0.634). In all other dental measurements (length of lower tooth row, length of lower molar tooth row and length of upper molariform tooth row), means were slightly higher in females of both age groups, but the

Table 2. Results of two-way ANOVA test. *P*-values (*p*) of significant effects of age and/or sex and interaction between them are highlighted in bold. For measurement abbreviations see Material and Methods.

	Age		Sex		Age × Sex Interactions	
	F value	*p*	F value	*p*	F value	*p*
HM	4.732	**0.031**	1.352	0.247	0.972	0.326
HP	0.218	0.641	0.026	0.873	0.583	0.446
Hm1	85.190	**< 0.001**	8.798	**0.004**	3.262	0.073
LI	100.030	**< 0.001**	12.090	**< 0.001**	6.207	**0.014**
LM	8.268	**0.005**	0.064	0.801	0.453	0.502
Lc1-m3	0.025	0.875	2.624	0.107	0.119	0.730
Lm1-m3	0.928	0.337	2.374	0.125	0.692	0.407
CW	7.622	**0.007**	9.047	**0.003**	3.705	0.057
ZB	2.980	0.086	0.372	0.543	0.308	0.580
IO	1.526	0.219	0.053	0.818	0.189	0.664
CB	7.029	**0.009**	0.023	0.880	0.958	0.330
LR	0.144	0.705	0.240	0.625	0.066	0.797
LN	23.200	**< 0.001**	3.256	0.073	2.932	0.089
LP4-M3	0.024	0.876	2.320	0.130	0.417	0.519

differences were not statistically significant (Table 2). Other measurements showed no sex differences. Sex has a significant effect only on the second principal component (F = 9.78, *p* = 0.002).

Discussion

Age variation

In his review about seasonal and age changes in shrews, Pucek (1970) states that the only cranial dimensions that change throughout the postnidal life of the common shrew are the depth and the breadth of the braincase. This statement can be attributed to the fact that previous studies focused mostly on seasonal changes of the braincase in relation to the Dehnel's phenomenon. Later, Homolka (1980) assessed fourteen skull measurements in shrews and found that four of them change during the postnidal life. Total length of the skull and length of the upper tooth row were significantly shorter in overwintered shrews compared with those in their first calendar year. As both measurements included the first upper incisor, the difference can be explained by continuous abrasion of this tooth during the individual's life. Length of the nasal bones was also found to decrease with age. Height of the braincase changed during the year in agreement with the Dehnel's phenomenon. There is an obvious discrepancy with our observations, although the measurements taken by Homolka are not always identical with those used in our work. We found significant age variation in seven of the fourteen measurements examined. Adults attained higher values than juveniles in six of these

measurements, while the opposite was true only for the length of the lower first incisor. The shortening of the lower incisor with advancing age in our sample is undoubtedly due to tooth wear. This conclusion is supported by the findings of Pankakoski (1989), who observed that in *Sorex araneus* and *S. minutus*, tooth wear is almost twice as fast in overwintered adults than in juveniles. Similarly, Stefen (2013) found that the length of the first lower incisor and length of the mandibular tooth row (including the first incisor) differ significantly in subadult and adult individuals.

The most frequently studied skull measurement is the condylobasal length (CB). There is no definitive consensus about its postnidal changes. The majority of studies (e.g. Dehnel 1949, Pucek 1955, Schubarth 1958, Hůrka 1986, Spitzenberger & Bauer 2001) did not find any significant differences between overwintered individuals and individuals in their first calendar year. On the other hand, Kubik (1951) found that overwintered individuals attain lower values of CB than youngs before overwintering. Homolka (1980) studied two populations of *S. araneus* living at markedly different elevations. He reported significantly higher CB in overwintered (adults trapped between April and November, in the second year of their life) than in juvenile shrews from the High Tatra Mountains, while in the lowland south Moravian population no such difference was present. Unfortunately, detailed studies of cranial intrapopulation variation in the common shrew are still very rare. As our analysis revealed statistically

significant differences in size and shape between the two age groups, age should be considered in future studies of *S. araneus* morphometric variation.

Sexual dimorphism
Most authors studying morphological variation of the *S. araneus* skull mentioned sexual dimorphism only briefly or did not discuss it at all (e.g. Churchfield 1990, Hausser et al. 1990, Churchfield & Searle 2008). Despite considerable sexual dimorphism in the postcranial skeleton (Dolgov 1961, 1985, Brown & Twigg 1970), it is generally accepted that the skull does not exhibit any dimorphism.

Early studies about morphological variation of the common shrew skull did not take sex into account (e.g. Dehnel 1949, Kubik 1951). Pucek (1955) found that overwintered females attain lower values in the height of the braincase than overwintered males. He assumed that this difference was caused by a later onset of reproductive activity in overwintered females, their gravidity, and consequent effect on their morphology. Schubarth (1958) confirmed Pucek's findings and also suggested that the condylobasal length attains somewhat higher values in males than in females. Surprisingly, even more recent studies did not group the specimens by sex (Homolka 1980) or found only negligible sex differences (Hůrka 1986, Yudin 1989, Spitzenberger & Bauer 2001, Mishta 2007, Poroshin et al. 2010, Zidarova 2015).

Significant sexual dimorphism was found in three of the fourteen measurements evaluated, and there was a significant effect of sex on the second principal component representing the shape. Higher values of the height of mandible measured below the first molar (Hm1) were revealed in males, which corresponds with the results of Poroshin et al. (2010). Although Poroshin et al. (2010) took this measurement in a slightly different manner, i.e. below the second molar, they found a statistically significant difference as well. The cranial width (CW) is well studied because of the changes it undergoes during winter (Dehnel's phenomenon). Greater values in overwintered individuals were found by Dehnel (1949), Kubik (1951), and Schubarth (1958). On the other hand, Pucek (1955) and Homolka (1980) did not detect any difference between the age groups. Our results (Table 1, Fig. 2) suggest that the matter is more complex. We found that only overwintered males have considerably broader CW than juveniles of both sexes, while overwintered females do not differ from either juvenile males or juvenile females. The discrepancy between these results could be explained by the fact that other authors did not divide their material by sex. The mean values of CW published by Spitzenberger & Bauer (2001), who divided their material into sex and age groups, agree with our results.

We found longer lower incisor (LI) in females than in males, where the difference was more pronounced in adults than in juveniles. Tooth wear is the principal cause of the gradual decrease of LI over the individual's life. Therefore, there are two possible explanations for the observed sex difference: different hardness of the teeth or different diet composition in males and females. As sex differences in the hardness of the *S. araneus* tooth enamel have not been found (Adamczewska-Andrzejewska 1966), we believe the effect of different diet in males and females is a more plausible explanation. White & Searle (2009) found a correlation between the mechanical potential of the mandible and climate factors in *S. araneus* females, but not in males. If the differences between males and females are contingent on climate conditions, diet, as a proxy for climate (e.g. Rudge 1968), can be a relevant explanation for our findings. Unfortunately, no information is currently available about potential sex differences in diet of the common shrew.

It should be noted that our results do not always agree with those reported in other studies of *S. araneus* populations from various parts of the species' range. Therefore, we hypothesise that age variation and sexual dimorphism in cranial morphology of the common shrew can be expressed to various degrees in different populations, depending on the environmental factors in different parts of its range.

Conclusions
Here, we show significant sex and age differences in several skull measurements in the studied South-Bohemian population of the common shrew. We investigated fourteen skull measurements and demonstrated changes in seven of them during the postnidal life of the individual. We found sexual dimorphism in three measurements. These facts should be considered in future studies about *Sorex* shrews.

Acknowledgements

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Supplementary online material
Table S1. Results of PCA. Factor loadings of the variables.
Table S2. Results of two-way ANOVA test on the first four principal components (PC). *P*-values (*p*) associated with significant effects of age and/or sex and interaction between them are highlighted in bold.
Table S3. Results of PCA. Eigenvalues of the correlation matrix (http://www.ivb.cz/folia_zoologica/supplemetarymaterials/novakova,_vohralik_tables_s1,_s2,_s3.docx).

Supplementary material
Table S1. Results of PCA. Factor loadings of the variables.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10	Factor 11	Factor 12	Factor 13	Factor 14
LM	−0.810241	−0.041582	0.023918	0.182738	0.027837	0.049973	−0.137269	−0.281312	0.153107	−0.134789	−0.109222	−0.389566	0.031306	−0.016826
HM	−0.656102	−0.104487	0.399586	0.339547	0.093328	0.156614	0.120022	−0.013844	−0.110101	−0.417638	0.152500	0.155623	−0.015089	0.039617
HP	−0.511976	−0.181904	0.470160	0.421585	0.127081	−0.313070	−0.212595	0.259941	0.055623	0.228326	0.140442	−0.058957	−0.024568	−0.006992
Lm1-m3	−0.698516	0.441445	−0.034958	0.214350	−0.287470	−0.055558	0.217713	−0.106594	0.043424	0.169974	0.052872	0.077122	0.278843	0.090958
Lc1-m3	−0.654912	0.517243	−0.030747	0.282998	−0.275047	0.012566	0.044243	−0.122001	0.027094	0.107549	−0.115611	0.127313	−0.282141	−0.091580
Hm1	−0.523796	−0.431095	−0.357069	0.074801	−0.333328	0.143927	−0.329672	−0.043037	−0.383257	0.071280	0.101360	0.018133	0.031797	−0.027631
LI	−0.064597	0.608068	0.446158	−0.423365	0.133686	−0.236948	−0.140768	−0.173803	−0.348922	−0.020209	−0.014945	−0.034007	0.014042	0.003783
CB	−0.801217	0.026946	−0.170644	−0.142780	0.451133	0.056629	−0.011478	−0.000775	0.055698	0.037057	0.000424	0.136890	0.124497	−0.249492
IO	−0.491902	−0.312992	0.193986	−0.588394	−0.277170	−0.079161	−0.085419	−0.179093	0.290958	−0.000096	0.238426	0.095811	−0.054970	0.008035
ZB	−0.649389	−0.409166	0.148458	−0.206223	−0.274522	−0.236572	0.059628	0.197700	−0.028407	−0.128788	−0.384368	0.066002	0.052455	−0.010056
CW	−0.618752	−0.398725	0.186556	−0.214989	0.150348	0.185811	0.441493	−0.013547	−0.186667	0.247994	0.020754	−0.139077	−0.089179	0.028975
LN	−0.524768	−0.159472	−0.594697	0.043602	0.355245	−0.400953	0.044641	−0.134761	−0.032360	−0.046291	0.017969	0.073553	−0.085060	0.142647
P4-M3	−0.551178	0.479637	−0.342173	−0.242835	−0.165072	−0.060108	0.106879	0.380290	−0.019851	−0.166439	0.186029	−0.194790	−0.039421	−0.031549
LR	−0.718239	0.276941	0.014819	−0.211407	0.226576	0.366586	−0.279818	0.169697	0.096124	0.080341	−0.124792	0.075633	−0.021827	0.179562

Table S2. Results of two-way ANOVA test on the first four principal components (PC). *P* values (*p*) associated with significant effects of age and/or sex and interaction between them are highlighted in bold.

	Effect	F value	*p*
PC1	Age	9.071068	**0.002996**
	Sex	0.134115	0.714661
	Interaction	0.210851	0.646691
PC2	Age	27.96902	**< 0.000001**
	Sex	9.78019	**0.002078**
	Interaction	0.59121	0.443026
PC3	Age	36.93360	**< 0.000001**
	Sex	0.00812	0.928295
	Interaction	0.05251	0.819027
PC4	Age	6.842402	**0.009708**
	Sex	0.019479	0.889168
	Interaction	0.027506	0.868473

Table S3. Results of PCA. Eigenvalues of the correlation matrix.

	Eigenvalue	% Total variance
1	5.660063	40.42902
2	2.024438	14.46027
3	1.348030	9.62878
4	1.050541	7.50386
5	0.901836	6.44169
6	0.587779	4.19842
7	0.537763	3.84117
8	0.407834	2.91310
9	0.395578	2.82556
10	0.322145	2.30104
11	0.259475	1.85340
12	0.237642	1.69745
13	0.190125	1.35803
14	0.076751	0.54822

II.   **Nováková Lucie**, Lázaro Javier, Kryštufek Boris & Vohralík Vladimír: Converse Bergmann´s rule drives the size pattern in the common shrew (*Sorex araneus*) in Europe. (manuskript)

1 **Converse Bergmann´s rule drives the size pattern in the common shrew (*Sorex araneus*)**
2 **in Europe**

3

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13

14 **Abstract**

15 The common shrew (*Sorex araneus*) is one of the most studied shrew species of the Western
16 Palaearctic. However, general rules forming its morphological variation still remain unclear.
17 On the large geographic scale there is a negative correlation between the body- and skull size
18 and longitude but on the regional scale such correlation is often missing; in fact, in some cases
19 the shrew size follows the opposite trend. We studied shrew mandibles using geometric
20 morphometrics and tested correlations of their size and shape with selected geographic and
21 climatic variables. We examined 1155 mandibles of the common shrew from 29 mostly Central
22 European localities and found significant inter-population differences in both size and shape.
23 Centroid size was positively correlated with longitude and negatively with latitude. We also
24 found significant correlations of centroid size with altitude and 13 climatic variables out of 19
25 examined. We confirmed a converse Bergmann´s rule in *Sorex araneus* in Europe along a
26 transect from the Baltic to the Adriatic. Altitude is an important factor influencing size of the
27 common shrew as large shrews were often found in higher elevations. Regarding mandible
28 shape, the Slovenian shrews were the most distant from all other studied populations. Influence
29 of altitude and precipitation on size of the common shrew was discussed. Future studies on
30 shrew variation should be aimed at altitudinal gradient within a single mountain massif.

32

1. Introduction

The common shrew (*Sorex araneus*) is the most widespread and abundant shrew species of the Western Palaearctic. Its distribution extends from the British Isles in the west to the Lake Baikal in the east, reaching the shores of the Arctic Ocean in the north and descending as far south as northern Greece. Throughout this vast area, its distribution is almost continuous and includes tundra, taiga, deciduous and mixed forest, marshes as well as forest-steppe habitats. Because of this wide occurrence the common shrew belongs among the most frequently studied mammals. So far, at least 40 subspecies have been described based mostly on skull and body dimensions of individual populations (Hutterer 2005). It is surprising that only few attempts have been made to find general rules forming morphological variation of this species. The first study based on relatively numerous material was published by Zalesky (1948) who concluded that in Central Europe common shrews increase in size from the north to the south and in Poland from the west to the east. However, he did not propose any explanation for this phenomenon. Following research on this topic has been devoted to the morphological differentiation between chromosomal races and to the validity of the Bergmann´s rule in explaining the size variation of the common shrew. Although the relationships between chromosomal races and their morphology still remain unclear (Stefen 2013, Shchipanov et al. 2014, Polly & Wójcik 2019), patterns of negative correlation between skull measurements and latitude (e.g., Polly 2007, Mishta 2007, Bobretsov et al. 2012) and altitude (Schipanov et al. 2011, Schipanov & Pavlova 2016) emerged from these studies. Similarly, a review study focused on Bergmann´s rule in five European *Sorex* species (including *S. araneus*) revealed a negative correlation between the condylobasal length of skull and latitude (Ochocińska & Taylor 2003).

A review of studies devoted to the relationship between *S. araneus* morphology and geographical as well as climatic variables was recently published by Mishta & Searle (2019). In general, on a large geographic scale there is a negative correlation between the body- and skull size and longitude. However, on a regional scale there seems to be no such correlation and even the opposite trend was found in some cases. Similarly, both trends were found in the relationship between shrew size and altitude in different regions. Although several hypotheses about the nature of the size variation of the common shrew have been suggested, none of them was unequivocally accepted so far. Therefore, the aim of our study is to describe the pattern of the morphological variation of the common shrew in the area covering Central Europe and part

the western Balkans and elucidate the cause of this morphological trend. We studied shrew mandibles using geometric morphometrics and tested dependence of their size and shape with selected geographic and climatic variables. Despite its obvious simplicity, the mandible is a remarkably flexible structure and actively reacts to various interactions and feedbacks by changing size and shape. Mandible evolved as a mosaic of elements and comprises several morphogenetic regions which show dissimilar rates of differentiation, composition, and function, making the mandible a highly informative structure.

2. Material and Methods

2.1 Material

We examined 1155 specimens of the common shrew (*Sorex araneus*) from 29 localities extending from the Baltic Sea to Northern Macedonia and from SW Germany to Bialowieża in NE Poland (Table 1, Fig. 3). Only individuals possessing intact mandible were examined. We used only young individuals (premature non-overwintered individuals) trapped between September and November of the year they were born to avoid differences due to sexual dimorphism, variation caused by Dehnel´s phenomenon, and ageing (Dehnel 1949, Lázaro et al. 2017, Nováková & Vohralík 2017). Skull materials are stored in the collections of Department of Zoology, Charles University (Prague, Czech Republic), Naturhistorisches Museum Wien (Wien, Austria), Hungarian Museum of Natural History (Budapest, Hungary), Museum für Naturkunde Berlin (Berlin, Germany), Max Planck Institute for Animal Behavior (Radolfzell, Germany), Slovenian Museum of Natural History (Ljubljana, Slovenia), Mammal Research Institute PAS (Bialowieża, Poland), Institute of Vertebrate Biology (Brno, Czech Republic) and Museum of Karlovy Vary (Karlovy Vary, Czech Republic), for a full list of specimens see Supplementary Material, Table S1.

We took pictures of the left mandible of all examined individuals. Mandibles were placed in the centre of the picture to avoid parallax effect. We placed the identical objective scale at every picture to calibrate the measurements. First, the order of all pictures was randomized using tpsUtil v. 3.2 (Rohlf 2015). Second, we placed 17 landmarks at every picture (Fig. 1, Table 2) using tpsDig2 v.3.2 (Rohlf 2015).

3

93   Information about exact location of trapping sites (GPS coordinates) were obtained from

94   museum databases. For each location we obtained 19 bioclimatic variables (Fick & Hijmans

95   2017).

96

97   2.2 Statistics

98   First, we performed generalised Procrustes analysis (GPA). In this step the redundant

99   information about position and rotation of landmark coordinates were removed (Zelditch et al.

100   2012). As an output of GPA we obtained two datasets – one containing Procrustes coordinates

101   (coordinates after superimposition) and the second containing size variable for all specimens

102   called centroid size. Centroid size (CS) represents overall size of the mandible and it is the

103   square root of the sum of squared distances of all the landmarks from the centroid of the

104   mandible (Zelditch et al. 2012).

105   Using dataset of Procrustes coordinates of each specimen we performed principal

106   component analysis (PCA; results not shown). To enhance the variation observed in the scatter

107   plot of PCA we performed canonical variate analysis (CVA). We visualise changes across main

108   axis of PCA and CVA as transformation grid. We test our dataset for an influence of allometry.

109   We did regression of Procrustes coordinates with centroid size as an explanation variable.

110   Residuals after this regression were used in another CVA.

111   We tested the influence of latitude, longitude, altitude and 19 bioclimatic variables on

112   the mandible size (CS). We created series of linear models with centroid size as a dependent

113   variable and latitude, longitude and their interception as an independent variable. We did the

114   same for altitude and bioclimatic variables.

115   We created a UPGMA cladogram of mean shapes of mandible from each location in

116   past v3 (Hammer et al. 2001). All other analyses were performed using R v. 3.4.2 (R Core Team

117   2013). We used MorphoJ v. 1.06 (Klingenberg 2011) for visualisation of shape changes.

118

3. Results

We found significant inter-population differences in both size and shape. We found a positive correlation of centroid size with longitude and negative correlation with latitude as well as a correlation with their interception (Table 3). CS was significantly correlated with altitude and 13 climatic variables from the total of 19 (summary in Table 4). Positive correlations were found with altitude, seasonality of temperature, and annual precipitation. On the other hand, we found a significant negative correlation with seasonality of precipitation and minimal temperature of the coldest month. Values of CS ordered according size did not reveal any straightforward geographical pattern leading in north-south or east-west direction (Fig. 2 and 3).

Using canonical variate analysis (CVA) we found clinal variation in shape leading from most of the studied European populations to Slovenian shrews (Fig. 4). The most distant was the population inhabiting Mt. Snežnik. However, this population was connected with other European populations by clearly transitional forms from central Slovenia (samples LJU, IGK). The most pronounced shape changes of the mandible were observed in the angle between *processus coronoideus* and *corpus mandibulae* (Fig. 5). The results of a linear regression of Procrustes coordinates on centroid size revealed allometry explaining 6% of variation in our material. When CVA was repeated on the allometry-free shape scores, differences between populations were negligible. UPGMA cladogram of mean shapes of mandible from all studied populations confirmed Slovenian populations as the most different from the rest ones (see Fig. 6).

4. Discussion

We found a converse Bergmann´s rule in *Sorex araneus* in Europe along a transect from the Baltic to the Adriatic. Centroid size of the mandible is positively correlated with skull size (own unpublished data) which in turn positively correlates with the overall individual body size. Thus, we can infer that significant negative correlation between centroid size and latitude ascertained in our material supports a converse Bergmann´s response in shrews. This conclusion is in concordance with previous studies, where the Eurasian *Sorex* shrews tend to follow the converse Bergmann´s rule (e.g., Ochocińska &Taylor 2003, Bobretsov et al. 2012).

149 On the large geographical scale covering almost the entire distributional area of the species,

150 Ochocińska &Taylor (2003) documented a highly significant correlation of condylobasal length

151 with latitude. Significant positive correlation of CS with longitude, which also emerged in our

152 study, is less unequivocal. A west to east size increase was observed by Zalesky (1948) in *S.*

153 *araneus* in Poland but was not retrieved by Bobretsov et al. (2012) in north-eastern European

154 Russia. Size of the common shrew also varies at a smaller scale and is influenced by a distance

155 from the sea. Shrews tend to be significantly bigger near the coast than inland (Frafjord 2008).

156 Therefore, it is possible that in central Europe the coast–inland climatic gradient is most steep

157 and as a result differences in size are more pronounced than differences observed in populations

158 from Russian plain, which are much more distant from the sea.

159 Altitude seems to be an important factor influencing size of the common shrew. Large

160 shrews were often found in higher elevations (e. g., Polly 2007, Schipanov et al. 2011). Our

161 material also retrieved positive correlation of CS with elevation, which accords the Bergmann´s

162 rule. However, the conclusion can be biased because majority of our material from the southern

163 Europe is from high altitudes (> 1000 m asl.). Polly and Wójcik (2019), who evaluated data

164 from more than 30 localities of *S. araneus* from altitudes varying between the sea level and

165 1700 m asl., found only a weak relationship between skull size and elevation. Unfortunately,

166 there is no study focused on altitudinal variation of *S. araneus* within the same mountain or

167 mountain range. Latitude, longitude and altitude are only surrogates to climatic factors, so we

168 must interpret our results with caution. In general, we also found negative correlations with

169 temperature and precipitation (see Tables 3 and 4).

170 Regarding mandible shape, the Slovenian shrews were the most distant from all other

171 populations. We found that most of the shape variation is due to allometry and therefore a

172 consequence of asynchronous growth of parts of mandible. The only real interpopulation

173 differentiation was in size. The Slovenian common shrews from Snežnik were bigger than those

174 from other studied populations and their overall appearance (e. g., fur colour) was visibly

175 different. To exclude the possibility of taxonomic confusion of the Snežnik population with *S.*

176 *arunchi* or *S. antinorii* known from north-east part of Italy, the species identity was verified

177 genetically (A. A. Bannikova, pers. comm.). An extraordinary size of these shrews cannot be

178 explained only by altitude (ca 1300 m asl.) since shrews from other southern moutains (Mt.

179 Pelister at ca 2200 m asl. and Popova Šapka at ca 1800 m asl.) were much smaller (Fig. 2).

180   Another plausible explanation could be a very high annual precipitation in Snežnik (ca
181   1860 mm) while in other studied populations it varies between ca 550 and 1390 mm. Other two
182   Slovenian populations (LJU, IGK) which are in size transitional between Snežnik and the rest
183   of studied populations (Fig. 2, 3 and 4), inhabit regions with the second highest annual
184   precipitation (1380 mm). This concurs an earlier observation that shrews are larger in regions
185   with high evapotranspiration and moister where certain invertebrates, their main food, are also
186   more abundant (cf. Churchfield 2002, Ochocińska &Taylor 2003).

187   An alternative explanation is that different phenotype of Slovenian shrew populations
188   is caused by different evolutionary history in comparison to the rest of examined populations.
189   Paleontological sites from the Last Glacial Maximum (23 000–16 000 BP) with temperate
190   mammal species are well documented for the region of Slovenia and its close neighbourhood
191   (Istrian and Slovenian Karst) (Sommer & Nadachowski 2006). Unfortunately, there is no
192   detailed phylogeographic study revealing the origin of the studied common shrew populations.
193   Therefore, a relict origin an the unique *S. araneus* population on Mt. Snežnik cannot be
194   excluded.

195   **5. Conclusions**

196   Although our results confirmed a converse Bergmann´s rule in *Sorex araneus* along a transect
197   from the Baltic to the Adriatic sea, some questions remain still unsolved. The next challenge
198   is to explain mutual influence of altitude and precipitation on shrew size. Therefore, future
199   study should be aimed on morphological variation in shrews collected in different altitudes of
200   a single mountain massif.

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286

Figure 1 Mandible of the common shrew with placed landmarks used in the study.

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289

Figure 2 Boxplots of CS

Figure 3 Map of localities of examined individuals of the common shrew. Points also correspond to mean centroid size of the population.
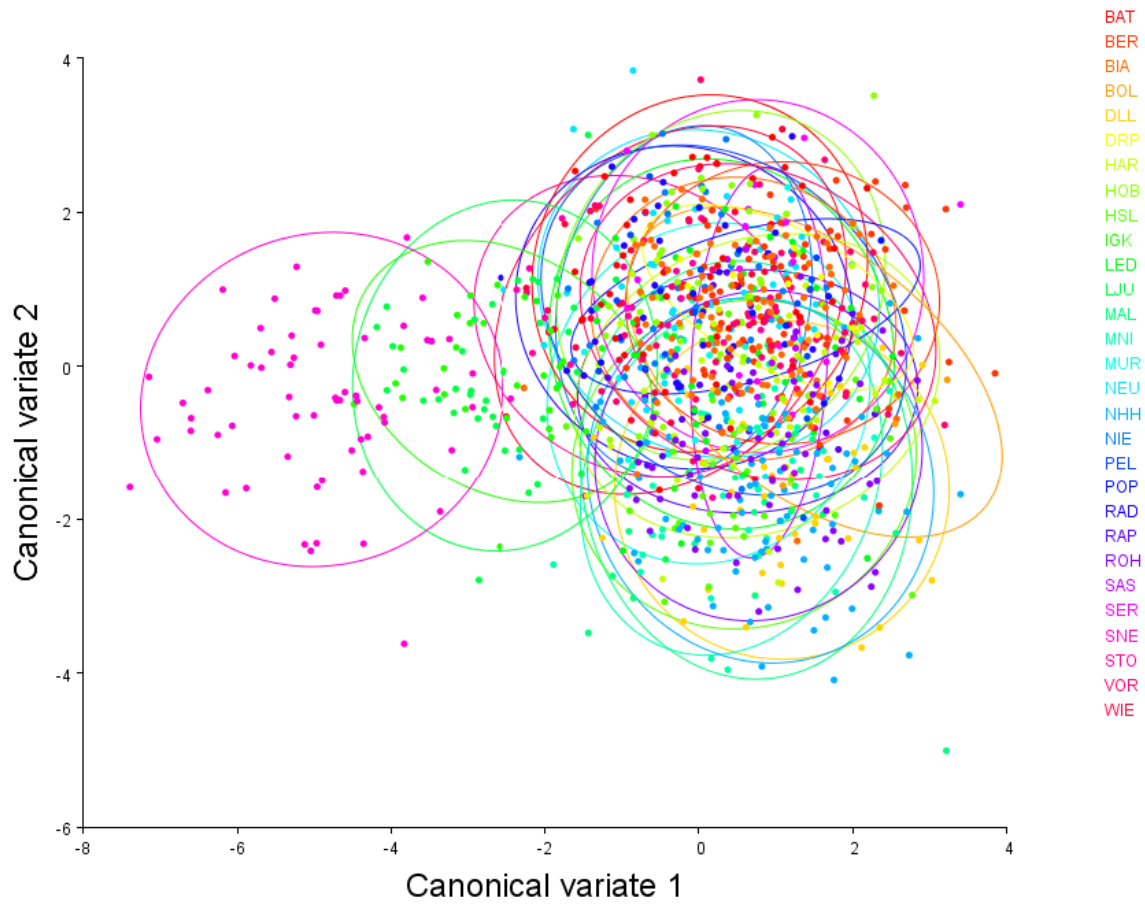
295

296   **Figure 4** Scatter plot of CVA based on Procrustes coordinates.

297

CV1

298

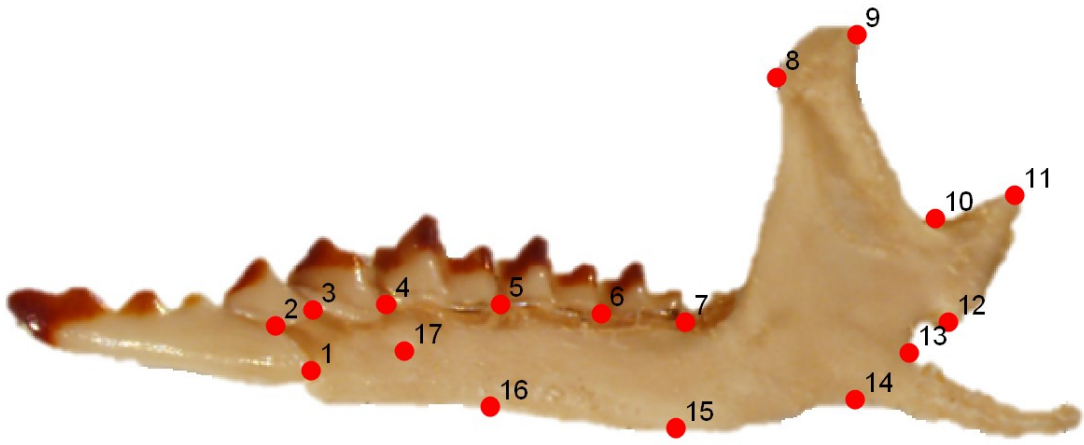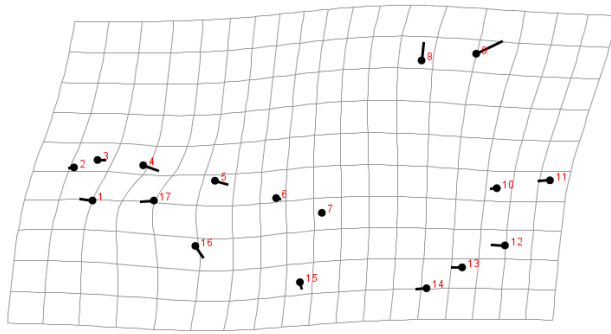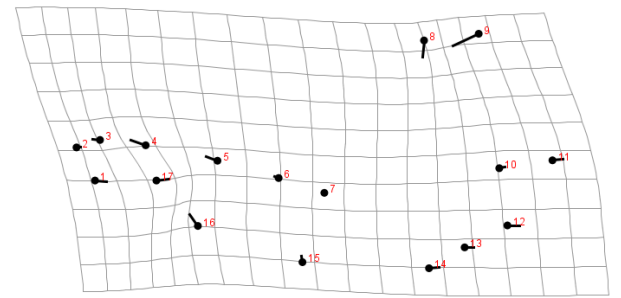**Figure 5** Shape changes of mandible across axis CV1, on scale on the left minus 10, on the right +10.

301
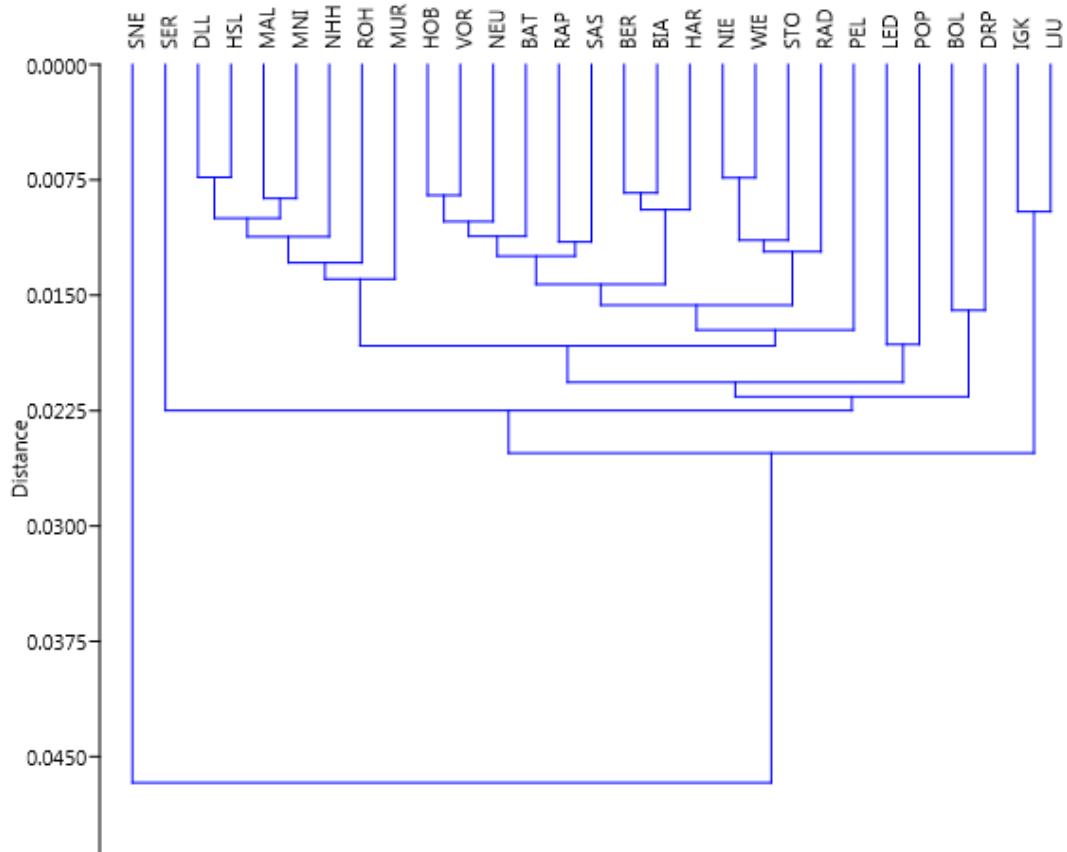
Figure 6 UPGMA cladogram of mean shapes of 29 studied populations of the common shrew
based on Euclidean distances between groups.

304

Table 1 Localities with number of mandibles of the common shrew.

state	locality	abbreviation	n	latitude	longitude
Austria	Murwald, Maria Buch-Feistritz, Murtal, Steiermark	MUR	14	47.249060	14.665683
Austria	Neusiedl am See, Neusiedl am See, Burgenland	NEU	47	47.744394	16.767704
Austria	Niederösterreich (mix loc)	NIE	39	48.495669	15.437084
Austria	Rappoldkogel, Reisstraße, Murtal, Steiermark	RAP	22	47.032470	14.919660
Austria	Biberhaufen, Stockerau, Korneuburg, Niederösterreich (+1)	STO	26	48.367205	16.237511
Austria	Wien	WIE	33	48.157457	16.246538
Czech Republic	Žofín, Novohradské hory	NHH	82	48.665756	14.704860
Czech Republic	Mnichovský potok	MNI	42	50.036808	12.776413
Czech Republic	Dolní Lazy	DLL	43	50.049288	12.630826
Czech Republic	Mariánské Lázně	MAL	31	49.966884	12.699850
Czech Republic	Horní Slavkov	HSL	38	50.138184	12.809088
Czech Republic	Lednice	LED	43	48.787260	16.802225
Germany	Radolfzell	RAD	39	47.765125	8.995220
Germany	Harz	HAR	57	51.762839	10.746784
Germany	Serrahn	SER	18	53.666257	12.344691
Germany	Sassnitz	SAS	10	54.532347	13.632502
Germany	Berlin und umgebung	BER	79	52.335783	13.841452
Hungary	Bátorliget	BAT	46	47.760231	22.262232
Hungary	Hajdú-Bihar (NP Hortobágy and surroundings)	HOB	49	47.576107	21.137994
Hungary	Kis-Balaton	VOR	72	46.643396	17.175224
Macedonia	Pelister	PEL	15	41.003375	21.187542
Macedonia	Šar planina, Popova Šapka	POP	10	42.012981	20.881360
Poland	Białowieża	BIA	91	52.710474	23.836230
Poland	Bolków	BOL	8	50.921872	16.108091
Poland	Drawsko Pomorskie	DRP	9	53.527422	15.806930
Slovakia	Liptovské Hole - Roháčská dolina	ROH	53	49.223920	19.726760

Slovenia	Snežnik, Sviščaki	SNE	65	45.594007	14.435756
Slovenia	Ig, Kremenica	IGK	31	45.958178	14.548306
Slovenia	Ljubljansko barje, Kozlerjeva gošča	LJU	43	46.008513	14.493595
	total number of mandibles		**1155**		

306

307 **Table 2** Description of used landmarks.

LANDMARK	DESCRIPTION
1	See Fig. 2 for definition
2	contact point of I_1, I_2 and the bone
3	contact point of I_2, P_4 and the bone
4	contact point of P_4, M_1 and the bone
5	contact point of M_1, M_2 and the bone
6	contact point of M_2, M_3 and the bone
7	posterior edge of tooth-row
8	anterior tip of the coronoid process
9	posterior tip of the coronoid process
10	maximum curvature on the curve between coronoid and articular processes
11	upper tip of the articular process
12	lower tip of the articular process
13	maximum curvature on the curve between articular and angular processes
14	maximum curvature on the curve between angular process and *corpus mandibulae*
15	See Fig. 2 for definition
16	See Fig. 2 for definition
17	*foramen mentale*

308

309 **Table 3** Summary of results of linear regression where centroid size is dependent variable;

310 longitude, latitude and their interaction are independent variables.

Response: Centroid.Size	Df	Sum Sq	Mean Sq	F value	Pr(>F)	correlation
longitude	1	0.01411	0.01411	7.7032	0.006	positive
latitude	1	0.59330	0.59330	323.9352	< 0.001	negative
longitude:latitude	1	0.34759	0.34759	189.7819	< 0.001	
Residuals	1151	2.10810	0.00183			

311

312   **Table 4** Summary of results of linear models for altitude and bioclimatic variables as
313   independent variable and centroid size as dependent variable.

Variable	F	df	Adj-R2	p	correlation
Altitude	32.49	1153	0.02656	< 0.001	positive
Annual Mean Temperature	0.0362	1153	-0.0008359	0.850	no
Mean Diurnal Range (Mean of monthly (max temp - min temp))	25.029	1153	0.0204	< 0.001	positive
Isothermality	21.44	1153	0.0174	< 0.001	positive
Temperature Seasonality (standard deviation *100)	4.1004	1153	0.002679	0.043	positive
Max Temperature of Warmest Month	3.8155	1153	0.002434	0.051	no
Min Temperature of Coldest Month	4.461	1153	0.00299	0.035	negative
Temperature Annual Range	9.206	1153	0.007061	0.002	positive
Mean Temperature of Wettest Quarter	100.7	1153	0.07953	< 0.001	negative
Mean Temperature of Driest Quarter	89.5	1153	0.07123	< 0.001	negative
Mean Temperature of Warmest Quarter	0.2818	1153	-0.0006227	0.596	no
Mean Temperature of Coldest Quarter	2.035	1153	0.0008964	0.154	no
Annual Precipitation	433.8	1153	0.2727	< 0.001	positive
Precipitation of Wettest Month	357.3	1153	0.2359	< 0.001	positive
Precipitation of Driest Month	325.6	1153	0.2195	< 0.001	positive
Precipitation Seasonality (Coefficient of Variation)	7.603	1153	0.005689	0.006	negative
Precipitation of Wettest Quarter	355.2	1153	0.2348	< 0.001	positive
Precipitation of Driest Quarter	336.2	1153	0.2251	< 0.001	positive
Precipitation of Warmest Quarter	194.8	1153	0.1438	< 0.001	positive
Precipitation of Coldest Quarter	241.9	1153	0.1727	< 0.001	positive

314

III.   Lázaro Javier, **Nováková Lucie**, Hertel Moritz, Taylor Jan R. E., Muturi Marion, Zub Karol & Dechmann Dina K. N.: Seasonal reversible size changes in small high-metabolic mammals exceed geographic variation - a review of Dehnel's Phenomenon in the common shrew. (manuscript)

1 **Title:**

2 Seasonal reversible size changes in small high-metabolic mammals exceed geographic variation -
3 a review of Dehnel's Phenomenon in the common shrew

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24   **Abstract**

25   1. Some small mammals showing Dehnel's phenomenon (DP) undergo a drastic decline in

26   braincase size and brain mass from summer to winter, followed by a regrowth in spring. This is

27   accompanied by a reorganization of the brain structure and size changes in other organs. These

28   changes might contribute to save energy during winter, but the link between DP and seasonality is

29   not clear yet. The magnitude of change varies between locations, but this inter-population variation

30   has not been explored. Elucidating trends and factors beneath DP variation can reveal its external

31   triggers and modulators and evolutionary drivers.

32   2. Here we explore geographic variation in DP intensity in *Sorex araneus*. We analyse the

33   geographic trends in change intensity and patterns of brain reorganization, to identify the

34   environmental variables that determine DP intensity.

35   3. We compiled literature on seasonal changes in braincase, brain and body mass, and tested the

36   correlations between the magnitude of change and geographic and climate variables. Also, we

37   collected new data on seasonal changes in brain regions to compare to published data.

38   4. In average, braincase decreases by 13% from summer to winter and regrows by 10% in spring.

39   For body mass the changes are 21/82%. These changes increase towards North-east, and

40   correlations to climate variables indicate a link between DP and seasonality. But decline and

41   regrowth phases show different environmental correlations, suggesting that they are independent

42   processes with different modulators.

43   5. The mean changes in brain mass were 21/10%, but we found no geographic trends. The seasonal

44   changes in brain regions found in Northern Poland described a reorganization of the brain structure

45   along the year, almost identical to the pattern already found in Southern Germany.

46   6. We discuss the techniques and criteria used for studying DP, as well as its potential presence in

47   other taxa and the importance of distinguish it from other kinds of seasonal variation.

48   **Key words:** brain mass, skull size, seasonal plasticity, brain regions, geographic patterns,

49   Soricidae

50   **Running head (50 char.):** Reviewing 70 years of Dehnel's Phenomenon

Introduction

To infer the adaptive value of phenotypic traits it is crucial to study functional correlations between both phenotypic and environmental variations. Many studies use phenotype variation between populations and individuals to address this. However, this approach neglects individual phenotypic flexibility, where an adult phenotype can still change in response to environmental change (Piersma & Drent 2003). A special case is life-stage cycling, i.e. seasonal phenotype changes along individuals´ life that are reversible. Studying this allows inferring mechanisms of adaptation to the environment as the changes are well marked and predictable.

An outstanding case of seasonal flexibility is the reversible but drastic morphological change called Dehnel's Phenomenon (DP) undergone by some small, short-lived, high-metabolic mammals. Best studied in the common shrew (*Sorex araneus*), DP entails a decrease in overall size, the size of the skull and other parts of the skeleton, but also the brain and many other organs and tissues in winter followed by a regrowth in spring (Dehnel 1949, Pucek 1965). Brain mass, for example, decreases up to 30% from summer to winter and increases again by 10-17% during the next spring and summer (Bielak & Pucek 1960, Lázaro et al. 2018a). Braincase height, often used as a proxy for the entire process, decreases up to 18% and regrows up to 15% (Crowcroft & Ingles 1959, Homolka 1980, Yaskin 1994). Importantly, DP causes not just a rescaling of the animal, but each organ and even each brain region shows a unique pattern in the direction and magnitude of change, resulting in several completely different phenotypes along the year (Yaskin 1994, Lázaro et al. 2018b). Other species of shrews, and as has recently been found, some mustelids, also show seasonal reversible shrinkage and regrowth at least of their skull and brain (Dechmann et al. 2017, LaPoint et al. 2017).

Species known to exhibit DP are small predators, with very high metabolic rates, which do not hibernate or migrate during winter (Taylor 1998, Ochocińska & Taylor 2005). They remain active and dependent on high quality food year-round and the plastic changes in skull and brain size are hypothesized to be a winter adaptation to save energy (Mezhzherin 1964, Pucek 1970, Yaskin 2011). While direct evidence of a link between the changes in overall size or specific organs such as the brain and individual survival are still lacking, reducing metabolically expensive organs, including the brain during winter, are thought to decrease overall energetic needs and thus food intake (Churchfield 1982). This would then compensate for the disadvantages of being small, such

3

81 as increasingly unfavorable volume to surface ratio in winter (Yom-Tov & Yom-Tov 2005). In

82 support of this, mass corrected energy consumption remains constant across seasons, which means

83 overall energy use of the size-decreased winter shrews is lower than in young summer animals and

84 especially the larger regrown spring individuals (Gębczyński 1965). In addition, although this

85 seasonal cycle occurs in every free-ranging individual studied to date, the intensity of size changes

86 is incredibly flexible. Captive shrews differed in the magnitude of seasonal change of skull size

87 when ambient temperature was manipulated (Lázaro et al. 2019).

88 Ambient conditions thus play an important role for DP, but whether that is as triggers or

89 evolutionary drivers or both, remains unclear. Braincase changes associated with DP in weasels

90 (*Mustela erminea* and *Mustela nivalis*) greatly vary in intensity and timing between populations at

91 different geographic locations (LaPoint et al. 2017). Previous studies on common shrews indicate

92 a greater winter decrease in skull and body size in Northeastern Europe compared to Southwestern

93 populations (Pucek 1970, Spitzenberger 2001). Similarly, the reorganization of brain structure also

94 greatly differs between two populations in Radolfzell (Southern Germany, Lázaro et al. 2018b)

95 and Russia (Yaskin 1994). This variation in seasonal size change between regions with milder and

96 harsher winter conditions supports the hypothesis that DP is a winter adaptation. However, a

97 review of latitudinal differences in seasonal body mass decline did not find any significant trend

98 (Ochocińska & Taylor 2003) and no direct or statistical comparison on braincase and brain size

99 has been made to date.

100 In this study we compiled all published work on DP to date and discuss progress made since the

101 last literature review in 1970 (Pucek 1970). First, we aimed to statistically test the influence of

102 geographic and climate trends on the magnitude of DP in *S. araneus*. We collected information

103 from those studies, which include changes in skull size and/or brain mass – and additionally

104 analyzed total body mass from those studies that include it – and explored correlations between

105 DP intensity and climatic and geographic variables. We added our own data on skull size, brain

106 mass and body mass from new populations in Poland and the Czech Republic to this dataset and

107 expected to find a geographical pattern that fits environmental factors related to seasonality, as

108 predicted by previous authors. Secondarily, we compiled information on DP in other species

109 compare results with *S. araneus*. Finally, we specifically investigated the variation between

110 populations in the structural changes within the brain associated with DP. We compared the

4

111 divergent results from southern Germany (Lázaro et al. 2018b) and Russia (Yaskin 1994) with new
112 data from a population in Poland, situated geographically between these two. We expected to find
113 intermediate values of structural change that would fit into a gradual, geographic pattern in this
114 Polish population. The aim of this review is to create an updated framework to study the
115 evolutionary aspects of this fascinating phenomenon.

Methods

Data compilation on magnitude of size changes from literature

118 We examined publications that report seasonal variation in skull size and/or brain mass in wild
119 populations of the common shrew (*Sorex araneus*). From those publications, we additionally used
120 values on total body mass when reported. For each study, we extracted the percentage of change
121 from the first summer size peak to the winter minimum (decrease), and from winter to the second
122 summer peak (regrowth) of each metric. For those studies, which did not directly provide an
123 estimation of the decrease and regrowth, we calculated it based on the published data. We
124 determined the first size peak as the month with the highest mean value for juveniles; the winter
125 minimum as the month with the lowest mean value for winter subadults; and the second size peak
126 as the month with highest mean value for adults. Summer juveniles are immature young
127 individuals born in late spring or summer; winter subadults are immature individuals, which are
128 ca. 6 months old; adults are individuals in spring and summer which were born the previous year
129 and have reached sexually maturity in spring. As *S. araneus* has a maximum life span of 13-18
130 months, there is no overlap of sexually mature individuals from two generations. When sample
131 size in a given month was low, we joined data from two or more consecutive months to determine
132 the corresponding size extreme. The amount of change was calculated as the difference between
133 mean values in the size extremes. To this data compilation from the literature we added own data
134 of decrease and regrowth in braincase height (BCH), brain mass and body mass from two new
135 populations (see below).

136 We determined geographic coordinates and altitude for all locations as geographic variables. We
137 extracted 19 bioclimatic variables from WorldClim Global Climate Data version 1.4 for all
138 locations (Hijmans et al. 2005). We used averaged values from data collected along 1960 - 1990
139 (see results section for details).

140　Following the criteria exposed above, we also compiled the same information on seasonal
141　morphological variation in other wild mammal species. However, the low number of publications
142　on DP in other species prevented any statistical analysis.

Skull dimension measurements from two museum collections

144　We included detailed data on skull dimensions and body mass of *S. araneus* from two populations:
145　Žofín, in the Novohradské hory mountain range (Czech Republic; 48.671838, 14.690402; new
146　data) from the dry collection deposited at the Department of Zoology at the Charles University in
147　Prague, which were collected from 1971 to 1977; and Białowieża National Park (Poland;
148　52.700000, 23.866667; Dechmann et al. 2017) at the Mammal Research Institute (Polish Academy
149　of Sciences). These specimens were collected in Białowieża and its surroundings, in the
150　Białowieża National Park in 1946-1947, and also preserved dry. We used a digital caliper (±0.01
151　mm) to obtain braincase height (BCH), from the tympanic rings to the dorsal surface of the
152　braincase, skull length (SKL), from the anteriormost projection of incisor 1 to the occipital
153　condyle, maximum braincase width (BCW) and lower mandible length, from the alveolus dentalis
154　of the incisor to the coronoid process. We focused our analyses on BCH as we had previously
155　found it to show the greatest change in response to DP (Lázaro et al. 2017, 2018a).

Collection of own additional data from two free-ranging populations in Poland and Germany

157　We also added data from two populations we collected ourselves: from Radolfzell, in the vicinity
158　of Lake Constance (Germany; latitude 47.766, longitude 8.994; published data from Lázaro et al.
159　(2018a)); and Gugny, in the Biebrza National Park (Poland, 53.347487, 22.589436; new data). We
160　captured shrews with wooden live traps (PPUH A. Marcinkiewicz, Rajgród, Poland) baited with
161　mealworms and checked at 2-h intervals. In Radolfzell we trapped monthly from December 2013
162　to July 2016. In Gugny we trapped at the estimated peak periods of the morphological change
163　cycle, in February, June and July 2014, May 2015 and May 2016. Immediately after capture,
164　shrews were weighed (±0.01 g) and carried to the laboratory where they were euthanized with
165　anaesthesia overdose (Isoflurane) and perfused transcardially with phosphate-buffered saline
166　(PBS) followed by 4% formaldehyde in PBS. Then we extracted the skull and used a digital caliper
167　(±0.01 mm) to obtain BCH, SLK, BCW as described above for museum specimens. After this, we
168　extracted the brain and weighed it (±0.001 g). We size corrected brain mass by the non-changing

169 maxillary tooth row length, which we obtained from post-mortem X-ray images of the skulls. This
170 size-correction factor had been used for brain and brain regions volume in previous work (see
171 Lázaro et al. (2017) for details).

172 We classified individuals as summer juvenile, winter subadult or adult based on the degree of
173 gonadal development, capture date and degree of tooth wear (Pankakoski 1989, Churchfield 1990).
174 For adults, sex can be directly determined visually. For immature individuals (juveniles and
175 subadults) for which we could not directly check the gonads during dissection, we determined sex
176 with a PCR-based gonosomal sexing method (Roos, DPZ Gottingen, unpublished). For this, we
177 extracted DNA from tail tip samples with a standard DNeasy kit (Qiagen, GmbH, Hilden).

178 Processing of brain tissue and calculation of brain region volumes

179 We quantified the volumes of brain regions based on 3D reconstructions of serial-sectioned tissue
180 as published in Lázaro et al. (2018b). Briefly, brains were cut sagittally in half and the two
181 hemispheres were weighed to the nearest 0.001 g. Then we fixated them for two weeks in PBS/4%
182 paraformaldehyde and then transferred them to PBS/0.1% sodium azide at 4 °C for long- term
183 storage. We used the left hemispheres for all volume reconstructions. Before sectioning, we
184 immersed the hemispheres in a series of PBS/10, 20 and 30% sucrose for cryoprotection. We cut
185 the tissue in the coronal plane on a freezing sliding microtone (Reichert- Jung Hn-40) to obtain
186 series of 30 μm-thick sections, of which we mounted every fifth section on microscope slides and
187 stained them with 0.5% cresyl violet. We measured the following brain regions: olfactory bulb,
188 neocortex, rhinal and piriform cortices, caudoputamen, amygdala, nucleus accumbens, thalamus,
189 hypothalamus, hippocampus, dentate gyrus, CA1, CA2, CA3, subiculum and cerebellum and the
190 total hemi-sphere (see Lázaro et al. (2018b) for details). To outline each region on the sections we
191 use the software Neurolucida (MBF Bioscience, Williston, VT, USA) and we applied the Cavalieri
192 principle to calculate the volume of each region based on the sum of the outlined areas multiplied
193 by the section thickness and inter-section distance. This calculation was made automatically in
194 Neurolucida Explorer. All data from Radolfzell were previously published in Lázaro et al. (2018b).

195 We accounted for the shrinkage of tissue occurring during the histological process by calculating
196 a correcting factor. This correcting factor was calculated for each brain as the quotient between the
197 original hemisphere volume – determined by dividing the fresh hemisphere mass by the specific

7

198 gravity of brain tissue (Stephan 1960) – and the final volume of the hemisphere as determined by

199 our reconstruction. The correction factor for each brain was then applied to the brain regions of

200 that specimen. We also size corrected brain region volumes by the upper tooth row obtained from

201 X-ray images (Lázaro et al. 2017).

Data analyses

Analyses of literature and own data

204 We analyzed the decrease and regrowth values of BCH, brain mass, and body mass from all

205 populations of *S. araneus* collected from the literature and the two new populations (Gugny and

206 Žofín). To explore geographical trends in the decrease and/or regrowth of BCH we fit two sets of

207 four linear models using percentage of decrease (in the first set of models) or regrowth (in the

208 second set) as response variable and a single dependent variable: longitude, latitude, altitude, or

209 the interaction latitude X longitude. We fit the same sets of linear models for brain mass and body

210 mass. Additionally, we used this same approach to analyze the geographical variation in overall

211 size (BCH, brain mass and body mass) in each of the age stages (juveniles, winter subadults and

212 adults). Similarly, we fit linear models for these response variables with each of the climate

213 variables.

Detailed analyses of morphological trends in four populations

215 To assess the differences in BCH, SKL, BCW, brain mass and body mass between DP stages and

216 locations, we used ANOVA for each of the five metrics. DP stage was treated as a factor with three

217 levels (summer juvenile, winter subadult, adult). We first assessed the effect of sex on our models

218 even though in our previous work we found no significant influence of sex on the seasonal changes

219 of these variables (Lázaro et al. 2017, 2018a). For each response variable (BCH, SKL, BCW, brain

220 mass and body mass) we compared two models using ANOVA: (M1) included season, location

221 and sex and their interactions as explanatory variables. We removed sex from the second model

222 (M2). We based our model selection on Akaike's information criterium (AIC) and chose M1 as

223 final model for each metric only if it revealed a lower AIC value and the difference between the

224 two models was significant. With the final model for each metric, we used Tukey tests to perform

225 pair-wise comparisons between the factor levels.

8

Analyses of seasonal variation in size of brain regions in Poland and Germany

227 To analyze the variation in volume of brain regions between DP stages, locations and sexes we

228 used ANOVA for each brain region separately, with size corrected volume of the brain region as

229 response variable and age, location and sex and their interactions as explanatory variables. Here,

230 we included sex in the model based on the significant effect we had previously found on the

231 seasonal changes of some brain regions in Radolfzell (Lázaro et al. 2018b). To tease apart the

232 influence of season, geographic difference, and sexual dimorphism, we did pair-wise comparisons

233 between the factors' levels using Tukey tests for multiple comparisons.

234 All analyses were performed in R 3.5.0 (R Core Team 2015).

235 **Results and discussion**

236 **Large scale geographic differences in magnitude of seasonal skull size and body mass**
237 **changes of *S. araneus***

238 Our results from the reviewed literature revealed large variation in DP intensity between

239 populations. The percentages of decrease and regrowth of BCH, brain mass and body mass in *S.*

240 *araneus* are compiled in the supplement review data file. Mean ±SD decrease in BCH from first

241 summer peak to winter minimum was 13.4±2.4% and mean regrowth from winter subadults to

242 overwintered adults was 10.3±2.8%. Percentage of BCH decrease was positively correlated with

243 latitude, longitude and their interaction (Fig. 1, see Table 1 for model summaries) but not with

244 altitude (Table 1). The large degree of variation in DP intensity between natural populations is

245 consistent with the great flexibility of skull changes observed in captive experiments in which

246 ambient temperature was manipulated (Lázaro et al. 2019). When analyzing BCH variation across

247 populations at each stage, we found a negative correlation of BCH with longitude and with the

248 interaction longitude X latitude at both the subadult and adult stage (P (sub.-long.)<0.05; P(sub.-

249 long.:lat.)<0.05; P (ad.-long.)<0.05; P(ad.-long.:lat.)<0.05;), but no trends with other variables.

250 This means that BCH of subadults and adults, but not of juveniles, decreased towards the

251 Northeast. We found a positive correlation in the intensity of BCH decrease with temperature

252 seasonality, temperature annual range, and precipitation seasonality, and a negative correlation

253 with isothermality and mean temperature of the driest quarter (Table 2). This suggests a link

254 between BCH decline and the intensity of seasonality, with a positive gradient towards more

255   continental climate. This is also supported by the correlations we found between DP intensity and

256   the climate variables associated with seasonality. As the decrease stage of DP has been postulated

257   to anticipate changes in climate and resource variability (which would be influenced by climate)

258   these patterns confirm the hypothesis that shrews shrink in winter to lower their energetic needs

259   and thus resource demands.

260   However, BCH regrowth was not correlated with any geographical variable (Table1) and was only

261   positively correlated with precipitation during the warmest quarter (Table 2). Striking is that body

262   mass as well as the size of crucial organs greatly exceeds juvenile mass, while the size of the brain

263   and skull only partially regrow (Pucek 1965). Similarly, energy expenditure is by far the largest in

264   these regrown adults. The most important investment shrews face during this period is

265   reproduction, associated with territory expansion, territorial fights, massive enlargement of the

266   testes in the males and the production of several large litters in the females (Vlasák 1996, 1998).

267   For both sexes this appears to be a more or less terminal investment as most individuals die shortly

268   after. Thus, regrowth with such a strong investment in mass might be driven by the demands of

269   reproduction. This would mean that decrease and regrowth phases have evolved under different

270   evolutionary pressures, which are caused and modulated by independent factors. Decrease

271   intensity would then mainly determined by the physiological limits of shrews, and the regrowth

272   by reproduction. The correlation only with precipitation might be linked to the availability of high

273   quality food during regrowth and reproduction since the abundance of the main prey of common

274   shrews, the common earthworm (Churchfield et al. 2012), is highly dependent on soil humidity.

275   Geographic patterns in seasonal body mass changes were similar to BCH. Across all reviewed

276   populations, body mass decreased by 21.2±6.2% and regrew by 81.9±18.2%. Similar to BCH, the

277   extent of body mass changes varied between populations in the analysis of literature data. We

278   found a positive correlation between body mass decrease with longitude, and with the interaction

279   of latitude X longitude, but not with altitude or in this case latitude alone (Fig. 1, Table 1). This

280   matches results from a previous study where no correlation between winter body mass decline was

281   found in *S. araneus* either (Ochocińska & Taylor 2003). Again matching results of skull measures,

282   body mass regrowth was not correlated with any geographical variable.

283   When comparing the three size peaks, there was no geographical pattern in juvenile or adult body

284   mass. However, there was a significant negative correlation of body mass of winter subadults with

latitude, longitude and their interaction, i.e. winter subadults had lower body mass towards Northeastern populations (P(long.)<0.05; P(lat.)<0.001; P(long.:lat.)<0.01). We found significant correlations between body mass decrease and most climate variables. In contrast, regrowth intensity was only negatively correlated with mean temperature during the driest quarter and, similar as in BCH, positively correlated with precipitation seasonality (Table 2). Again these patterns support the hypothesis that different evolutionary drivers are responsible for the decrease - shrinking as an adaptation to save energy during cold periods with low resource availability, and the increase - growing a large body size well adapted for territory defense and to maximize reproductive output especially in females.

While the changes in body mass we describe are dramatic, seasonal fluctuations in body mass are common in mammals. For example, North American beavers (Castor canadensis) lose 9-12% of their body mass during autumn and winter, mainly because of the metabolic use of their fat stores (Smith & Jenkins 1997). During hibernation, marmots can lose 32% of their body mass (Lenihan & Vuren 1996), and hedgehogs 15-28% (Haigh et al. 2012). None of these species exhibit the changes in skull and brain size, which are characteristic of DP. Thus, changes in body mass are not exclusive of DP and should only be used to describe DP in combination with other variables (see also general remarks below).

Differences in seasonal skull dimensions and body mass between four populations of the common shrew

As previous studies had also found (Dehnel 1949, Lázaro et al. 2017, 2018a), BCH showed the strongest size changes in all populations. Thus, we show only results for BCH here. Results for SKL and BCW can be found in the supplementary material (Table S1).

In our own previous work we used mandible length as a factor to correct for individual size variation as we found it to remain constant throughout the shrews' lifespan once summer juveniles are fully grown at our study site in Southern Germany (Lázaro et al. 2017). However, when looking at three additional populations (Žofín, Gugny and Bialowieza) we found that mandible length varied between seasons (d.f.=186, adj. R2=0.19, F=5.3, P(seas.)<0.05, P(loc.)<0.001, P(seas.:loc.)>0.1). As results for size-corrected and absolute values did not significantly differ in Radolfzell, we ran all analyses for the comparison of the four populations with absolute values.

314   First we tested for the effect of sex, but the comparison between models revealed no significant

315   effect of sex and its interactions on BCH for all locations (AIC (M1)=-79.2, AIC(M2)=-67.8;

316   ANOVA, P>0.5). Thus, we excluded sex from further comparisons of skull dimensions. This is

317   interesting, as even though differences in behavior and energetic pressure should exist particularly

318   during reproduction in the adults, and some sexual dimorphism was found in mandible

319   morphology *S. araneus* (Nováková & Vohralík 2017).

320   In the final model M2 (d.f.=200, adj. R2=0.78, F(season)=155.7, F(location)=146.6, F(interaction

321   seasons:location)=1.3), there was a difference between seasons and locations at the factor level

322   (P<0.001 both) but not their interaction (P>0.1). The Tukey test revealed a decrease in BCH from

323   summer juveniles to winter subadults (P<0.001) and increase from winter subadults to adults

324   (P<0.001) in each location (Fig. 2, Table 3). For all seasons combined, BCH values from Gugny

325   were the highest, followed by Radolfzell, Žofín and Białowieża (P<0.05 in all pair-wise

326   comparisons, Table 3). Thus, shrews from the four populations differed in size, but the magnitude

327   of DP did not. Our analyses of data from the literature confirmed a more pronounced decline in

328   BCH towards Northeastern populations at a large geographical scale. However, our review also

329   revealed large levels of variation in winter size decline between populations within small areas

330   (e.g. northern Germany(Schubarth 1958)) and similar decline values in widely separated

331   populations (e.g. southern Germany and central Finland (Skaren 1964, Lázaro et al. 2018a)). Our

332   four focal populations did not follow the predicted pattern but they are all situated in central Europe

333   and habitat differences might not be strong enough to cause the variation observed at a larger scale.

334   Interesting is also that size, as measured by BCH did not follow the expected pattern either. The

335   common shrew is smaller with increasing latitude in direct contradiction to Bergmann's rule,

336   however the two neighboring Polish populations differed in size more than Gugny (Northeastern

337   Poland) and Radolfzell (Southern Germany), which were almost identical.

338   Also similar to BCH results, we found few differences in body mass between the more closely

339   investigated populations (Radolfzell, Gugny and Žofín). Again we found no significant effect of

340   sex on body mass variation between seasons (AIC (M1)=290.3 ,AIC(M2)=293.6 ;ANOVA, P>0.1)

341   and pooled data of males and females in all analyses. Body mass differed significantly between

342   seasons and locations at both factor and interaction levels (M2, d.f.=116, adj. R2=0.88,

343   F(seas.)=424.2,   F(loc.)=13.8,   F(seas.:loc.)=2.8,   P(seas.)<0.001,   P(loc.)>0.001,

344   P(seas.:loc.)>0.05). All three populations decreased from summer juvenile to winter subadult
345   followed by a pronounced regrowth to adult (Table 3, Fig. 3, Tukey test, P<0.001 for all
346   populations). Body mass was similar in juveniles and adults in all populations, but winter subadults
347   from Žofín were lighter (P<0.001). Žofín is the only high-altitude population in our analysis.
348   Mountain populations suffer harsher winter conditions and therefore we would expect DP to be
349   stronger in shrews at higher altitudes. Thus, the stronger body mass decline found in Žofín supports
350   DP as a seasonal adaptation. However, we do not see a matching difference in BCH decline. This
351   might mean that changes in body mass are more sensitive to local environmental differences and/or
352   current conditions. For example, there is the little evidence for winter body mass decline in Norway
353   (Frafjord 2008), but a 27% decline found at similar latitudes in Finland (Hyvärinen & Heikura
354   1971). In fact, especially given that data from the various sites were collected during completely
355   different years, seasonal changes in body mass may have resulted from other causes independent
356   from DP, for example, winter malnutrition non-adaptive changes.

357   **Geographic differences in seasonal brain mass changes**

358   Literature on seasonal changes in mammalian brain size was scarce, but average brain mass
359   (supplementary data) decreased by 20.9±5.6% from summer to winter and regrew by 10.0±4.2%
360   to adult size. This is the most remarkable aspect of Dehnel's phenomenon. The size of the
361   mammalian brain, once fully grown is usually more or less fixe and changes of this magnitude are
362   unparalleled. Only the brain of some songbirds reversibly changes by similar magnitudes
363   (Nottebohm 1981, Tramontin et al. 1998). Experimentally induced changes in brain size, by
364   starving or other environmental manipulations, are usually less than 5% (Bedi & Bhide 1988). In
365   humans, brain size increases during ontogeny, reaching a peak at the age of 20; then, after 45-50
366   years of age it undergoes a progressive, unidirectional decline of 11% over the next 40 years, as a
367   result of ageing (Dekaban & Sadowsky 1978).

368   When analyzing the literature data we did find some variation between populations. However, the
369   intensity of both decrease and regrowth of brain mass were not correlated with any geographical
370   or climate variable (Tables 1 and 2).

371   When looking at our own data from Gugny and Radolfzell in more detail, we again found no
372   significant effect of sex on the variation of corrected brain mass in our model comparison (AIC

373   (M1)=-509.6 , AIC(M2)=-509.7 ;ANOVA, P>0.1). We did find significant variation between

374   seasons at the factor level in the final model M2, but not between locations and their interaction

375   (d.f.=50, adj. R2=0.45, F(seas.)=22.5, F(loc.)=0.5, F(seas.:loc.)=2.1, P(seas.)<0.001, P(loc.)>0.5,

376   P(seas.:loc.)>0.1). However there was no difference in absolute brain mass at any of the age stages

377   that could be explained by geographical variables either (P>0.1 for all models). In both locations

378   there was a significant but similar decrease from summer juvenile to winter subadult (Tukey test,

379   P<0.001). Surprisingly we found no significant regrowth from winter subadult to adult (P>0.1).

380   Thus, corrected brain mass was similar at every stage in both populations (Table 3, Fig. 4). This

381   lack of spring regrowth in brain mass in contradicts previous research, which reported a significant

382   increase in brain mass from winter to summer in all studied populations of common shrews (Bielak

383   & Pucek 1960, Pucek 1970, Yaskin 1994, Lázaro et al. 2018a). In fact, in our own previous study

384   of the Radolfzell population we found a significant regrowth in brain mass, with a maximum

385   during July-August (Lázaro et al. 2018a). The adult brains used for the present analyses were

386   collected earlier (May-July), especially in Gugny, where most adults were collected in May when

387   body mass regrowth peaks and which is commonly used for measurements of the regrowth peak.

388   Thus, the adults included here probably had not completed brain regrowth yet. This enhances the

389   importance of timing in data collection and stage definition for DP studies. An accurate estimation

390   of the peaks and minimum of the cycle is critical for the quantification of the change. Probably

391   most studies of DP are not reporting exact maxima and minima, and consequently all values on

392   change intensity presented here are timed with spring body mass maxima and thus underestimates

393   of the actual change in brain mass. This must be considered when studying DP intensity as well as

394   the cycle phenology.

395   Striking in all the combined results above is the discrepancy between variables. Changes in BCH

396   were not paralleled by changes in brain mass, unlike in previous studies (Bielak & Pucek 1960,

397   Lázaro et al. 2018a). The geographical patterns we found in BCH decrease, were not reflected by

398   patterns in brain mass changes. This is probably due to the small sample size of brain mass data.

399   Only few studies (10 publications about the common shrew, the most studied species) have

400   investigated the seasonal changes in brain mass.

401   **Comparing seasonal changes in brain organization in two populations**

402   As already previously published for the Radolfzell population, the different brain regions from

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403   Gugny confirm different patterns of decrease and/or regrowth (or lack of change) in the volume of

404   each brain region (Fig. 5). As each region contributed to the overall decrease/regrowth of brain

405   volume to a different extent, we observed a seasonal change in the organization of the brain.

406   However, the pattern of decrease and regrowth was similar at both locations in most brain regions,

407   leading to an almost identical seasonal variation in Radolfzell and Gugny. Olfactory bulbs of

408   females but not males significantly decreased from summer juveniles to winter subadults in both

409   locations ($P<0.05$) reflected by a significant interaction of age and sex ($F=4.2$, $P<0.05$), but we

410   found no difference between Radolfzell and Gugny at neither factor nor interaction level ($P>0.1$).

411   The only other brain region where the pattern differed between the sexes was the cerebellum but

412   only in Radolfzell (see also Lázaro et al. (2018b)). The cerebellum did not vary seasonally at either

413   location ($P>0.5$). However, subadult winter females in Radolfzell had larger cerebelli than males,

414   while in Gugny we did not find this sexual dimorphism. Changes for all other brain regions are

415   discussed for males and females together. Volume of the neocortex significantly declined from

416   summer juveniles to winter subadults at both locations ($P<0.001$). We found that summer juveniles

417   have larger neocortex in Gugny ($P<0.01$) but we found no difference in winter, meaning that there

418   was a more pronounced decrease in Gugny. We did not find a difference in neocortex volumes

419   between winter subadults and adults from both locations. The rhinal and piriform cortices

420   decreased their volume from summer to winter ($P<0.01$) and did not increase in adults, with no

421   difference between locations. Also, overall striatal volume decreased from summer juveniles to

422   winter ($P<0.001$), but did not increase in adults ($P>0.5$). Again, there was no difference between

423   locations. Within the striatum, this pattern was repeated in the caudoputamen (P (juv-sub)<0.001;

424   P (sub-ad)>0.5) and amygdala (P (juv-sub)<0.05; P(sub-ad)>0.1), while the nucleus accumbens

425   did not significantly differ in any of the variables. The overall volume of the hippocampus

426   decreased from summer to winter ($P<0.05$) and did not regrow in adults. There was no difference

427   between locations. Within the hippocampus, volume decrease was only found in CA2 (P (juv-

428   sub)<0.05). Both the thalamus and hypothalamus decreased and regrew significantly, with no

429   difference between locations (thalamus: P (juv-sub)<0.001; P (sub-ad)<0.05); hypothalamus: (P

430   (juv-sub)<0.001; P(sub-ad)<0.001).

431   The main finding here is that brain organization changes seasonally, and that these changes are

432   consistent in Radolfzell and Gugny. As previously shown for Radolfzell (Lázaro et al. 2018b), the

433   shrinkage and regrowth phases differ between brain structures. Thus, each brain region makes a

434 different contribution to the seasonal changes in brain size, giving rise to a marked re-organization
435 of the brain structure along individuals' life. The seasonal changes in each brain region in Gugny
436 are remarkably similar to the variation observed in Radolfzell, with the exception of a slight
437 difference in neocortex winter decrease – more accentuated in Gugny – and a quite different pattern
438 in the Cerebellum. However, the lack of brain mass increase in spring in Gugny may be due to
439 early sampling and the results in Lázaro et al. (2018b) from Radolfzell might describe these actual
440 patterns better.

441 Interestingly, the structural changes described in Russia (Yaskin 1994) largely differ from both
442 Radolfzell and Gugny. The only brain region with a similar pattern is the neocortex, which is the
443 structure that undergoes the greatest winter decline in all three populations – 37% decline in Russia
444 and Gugny, 28% in Radolfzell – although this is followed by a 18% regrowth in Russia, which we
445 did not observe in the other populations. Russian shrews undergo a shrinkage/regrowth in mass of
446 the paleocortex of 28/12% respectively, more pronounced than the intermediate values in the
447 correspondent regions – rhinal and piriform cortices – in Gugny (21/6%), and the lower changes
448 in Radolfzell (18/4%). This is the only brain structure that matches our expectation of a geographic
449 and/or environmental gradient, with the Polish population intermediate between Russia and
450 Germany. Hippocampal changes are much higher in Russia (29/33%) than in Gugny (10/5%) and
451 Radolfzell (10/8%), while olfactory bulbs, which did not change seasonally in Russia, showed
452 strong changes both in Radolfzell (14/14%) and Gugny (24/12%). These incongruences refute the
453 hypothesis of a simple linear geographic trend. Instead, the differences in brain structure between
454 populations and seasons might reflect local adaptations to specific environmental features.
455 Nevertheless, we must also point differences in methods used, while we use volumetric estimations
456 derived tracing brain regions in fixed, sectioned and stained sections, the method used in Russia
457 was mass of dissected tissue (Yaskin 1994). Thus, a methodological bias might influence the
458 overall observed variation.

459 **Dehnel's phenomenon in other species and general remarks**

460 *Sorex araneus* is a model species for DP studies, however, it is not the only species showing DP
461 and, in fact, not showing the most extreme changes. We found literature on 16 mammalian species
462 – including *S. araneus* – reporting seasonal variation in braincase and/or brain size (Table S2,
463 supplementary material). Seven of these species belong to the genus *Sorex* and 10 of them are

464 shrews (Soricidae). The most profound seasonal changes were reported from *Sorex minutus*: it

465 undergoes a winter decrease and spring regrowth in BCH of 19.1% and 15.5%, respectively (Kubik

466 1951); and a 34.3% decrease and 20.3% regrowth in brain mass (Caboń 1956).

467 Most of species showing DP are soricids and small mustelids. They have in common that are small,

468 short-lived predators with fast metabolism, which are unable to use torpor or hibernate and which

469 mostly delay reproduction to the following spring. Thus, the presence of DP in these two

470 phylogenetically distant groups might be a convergent adaptation to winter under similar

471 conditions (Dechmann et al. 2017). This is confirmed by additional reports of decline in braincase

472 and brain size in captive mustelids. Brains of captive ferrets (*Mustela putorius*) shrink by 11-19%

473 during 10 months after a postnatal growth peak (Apfelbach & Kruska 1979, Weiler 1992). A

474 similar decrease of 14-18% in brain mass was observed in mink from fur farms (*Mustela vison*)

475 (Kruska 1977) here also followed by 17% regrowth in adults (Kruska 1993). However, we

476 excluded these studies from our species list because the changes were not clearly linked to

477 seasonality, and there is a known decreasing effect of domestication on brain size (Kruska 1993).

478 There was one exception to the species pattern: arvicoline voles (Rodentia) also show seasonal

479 morphological variation (Yaskin 1984, 2011, 2013), but they do not share the physiological

480 characteristics and food requirements of soricids and mustelids. However, part of the definition of

481 DP is that the morphological change happens at the individual level. A change in average size of

482 skull or brain at the population level does not necessarily reflect individual size changes. For

483 example, selective mortality of large individuals during summer and autumn can lead to a decrease

484 in mean population size towards winter in voles and weasels (Szafrańska et al. 2013, Zub et al.

485 2014). In contrast to shrews, which reproduce only in their second summer, some species breed

486 year round. Thus, variations similar to DP could be also caused by seasonal size differences in

487 cohorts, that is, animals born in autumn and winter might achieve smaller final size than those born

488 in spring and summer, as is the case in some rodents and non soricine shrews (Schwarz et al. 1964,

489 Dapson 1968, Brown 1973, Markowski & Ostbye 1992). Confounding DP and a seasonal cohort

490 effect in Blarina brevicaudaI wrongly led to reject the existence of DP (Dapson 1968). In addition,

491 a mean decline can be caused by emigration of large individuals or recruitment of small ones

492 (Iverson & Turner 1974). After any of these processes, the decline might be followed by an

493 increase in mean size, caused by the inverted process or simply by continued individual growth,

494   which then cannot be considered a "re-growth". Size-corrected analyses, such as Dechmann et al.

495   (2017), LaPoint et al. (2017) and Lázaro et al. (2018a) are necessary to account for individual size

496   variation and describe relative changes in the size of the brain. The only species for which DP in

497   the skull and thus brain has been confirmed at the individual level is *S. araneus* (Lázaro et al.

498   2017). For this species, in Radolfzell the mean BCH decreased by 12% from July to February at

499   the population level (Lázaro et al. 2018a); but in that same population some individuals decreased

500   by 15-20% along that period (Lázaro et al. 2017), indicating that the estimations at the population

501   level might be biased by the factors mentioned above. Thus, when studying DP we must carefully

502   choose the approach and methods.

503   This once more also emphasizes the importance of using mass only in combination with other

504   variables to describe DP. Individual loss in body mass from summer to winter can have different

505   causes, predominantly it can be a direct consequence of lack of resources in winter. Many species

506   store fat resulting in a weight peak in late summer, followed by a decline along autumn and winter

507   as they use it up. In contrast to the anticipatory shrinking of the shrew, which also includes the

508   skeleton and many major organs, this body mass decrease is therefore not adaptive but

509   consequential. Common shrews in captivity reduce food intake during winter and decrease both

510   body mass and BCH also when provided with food ad libitum (Churchfield 1982, Lázaro et al.

511   2019). This indicates that body mass decline associated with DP is indeed adaptive. The two kinds

512   of body mass changes – consequential vs. facultative – are regulated by different physiological

513   processes, triggered and modulated by different external zeitgebers and are ultimately the result of

514   different evolutionary drivers (Hyvärinen 1984). Thus, they must be studied under separated

515   theoretical frameworks not to be confounded. We suggest that individual changes in skull

516   dimensions and brain mass are the most distinctive features of the morphological changes

517   associated with DP. Until the size changes of other organs have been better described for various

518   populations, we recommend using the skull and brain as the primary metrics to verify and measure

519   DP.

520   As important as choosing the right morphological trait to measure is the correct timing of

521   measurements. As indicated by our brain size results from Gugny choosing the wrong timing may

522   profoundly affect how DP is described in a given study. To date, the phenology of DP has not been

523   investigated. To the best of our knowledge, based on our own data and the information collected

524 from literature, the time of the year at which each stage of the DP takes place may vary between

525 populations. In the common shrew the first size peak in the summer juveniles occurs between June

526 and August; the minimum in winter subadults has been reported between December and March;

527 and the second peak, in sexually mature adults, is reached between May and August. In addition,

528 the timing at each site may differ for body mass and skull/brain measures. Also, the duration of

529 both decrease and regrowth phases have a strong impact on individuals' biology, as it determines

530 the rate of tissue shrinkage or regeneration. Viktorov (1967) suggested a possible geographic trend

531 in DP phenology: the braincase regrowth phase tends to shorten from western (UK) to eastern

532 (Russia) Europe, in contrast to the rate of regrowth which increases towards eastern populations.

533 Studying the specific timing of each peak and minimum in the each population might reveal

534 correlations with current environmental factors and therefore provide more information on the

535 triggers and evolutionary drivers of DP. Such added knowledge of the exact timing of the change

536 of each tissue (bone, brain region or organ) in conjunction with studies of gene expression and the

537 detailed mechanisms involved will be important to truly interpret the adaptive value of DP. For

538 example, the fact that the brain is largest in young dispersing juveniles and then only partially

539 regrows in reproductive adults, which instead invest in larger body mass suggests that different

540 drivers lead to the shrinking and the regrowth but only a detailed and holistic quantification of the

541 costs and functions of various tissues at each stage will allow us to answer this. Perhaps then, we

542 can understand more general questions, as why soricine shrews and small mustelids pursue the

543 risky strategy of reproducing only so close to the end of their brief lifespan.

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680

Figure legends
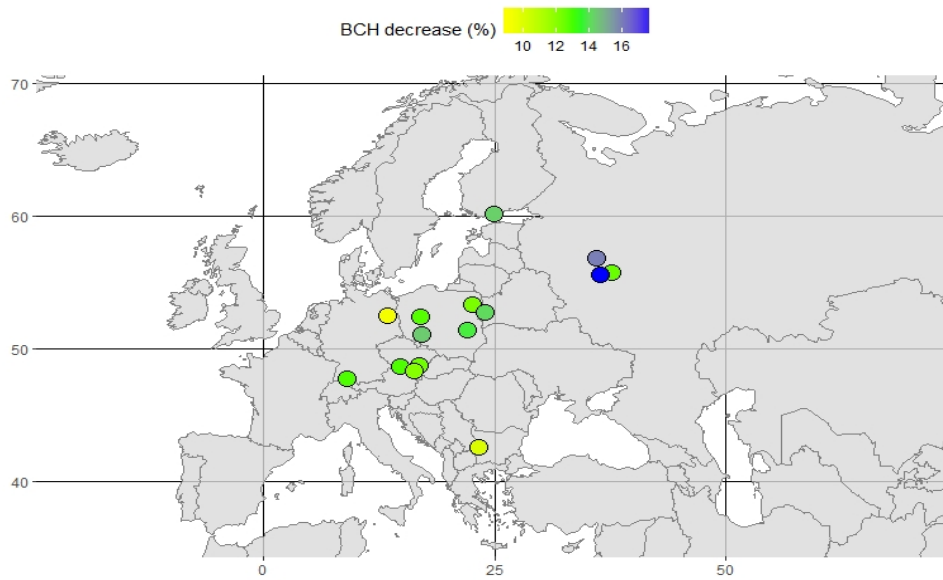
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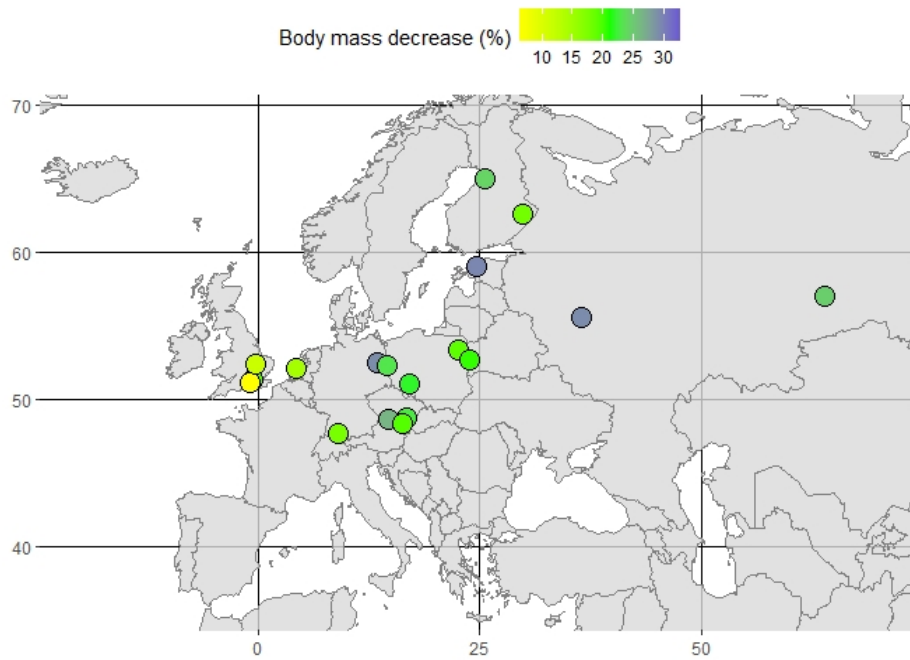
683

684 **Figure 1.** Intensity (%) of decrease in BCH and body mass in different populations of common
685 shrew across Europe.

686

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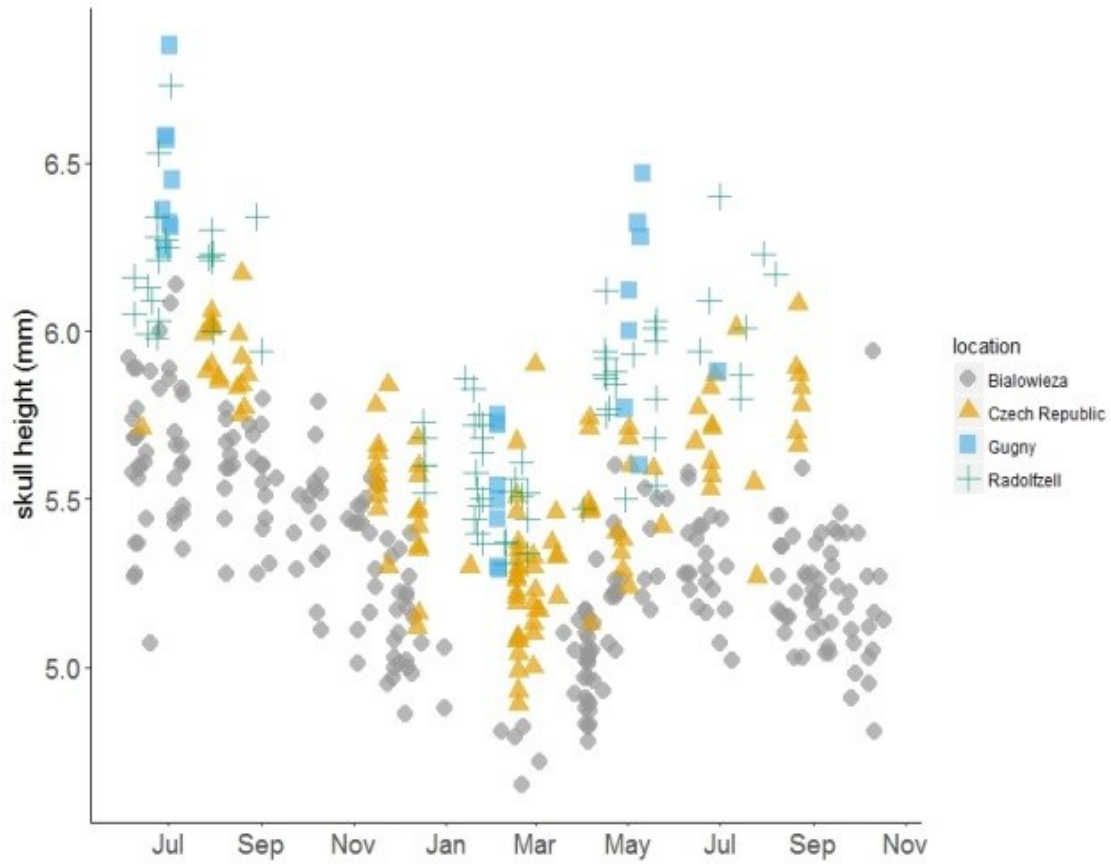
Figure 2. Seasonal variation in braincase height in the four populations analyzed in detail.

26
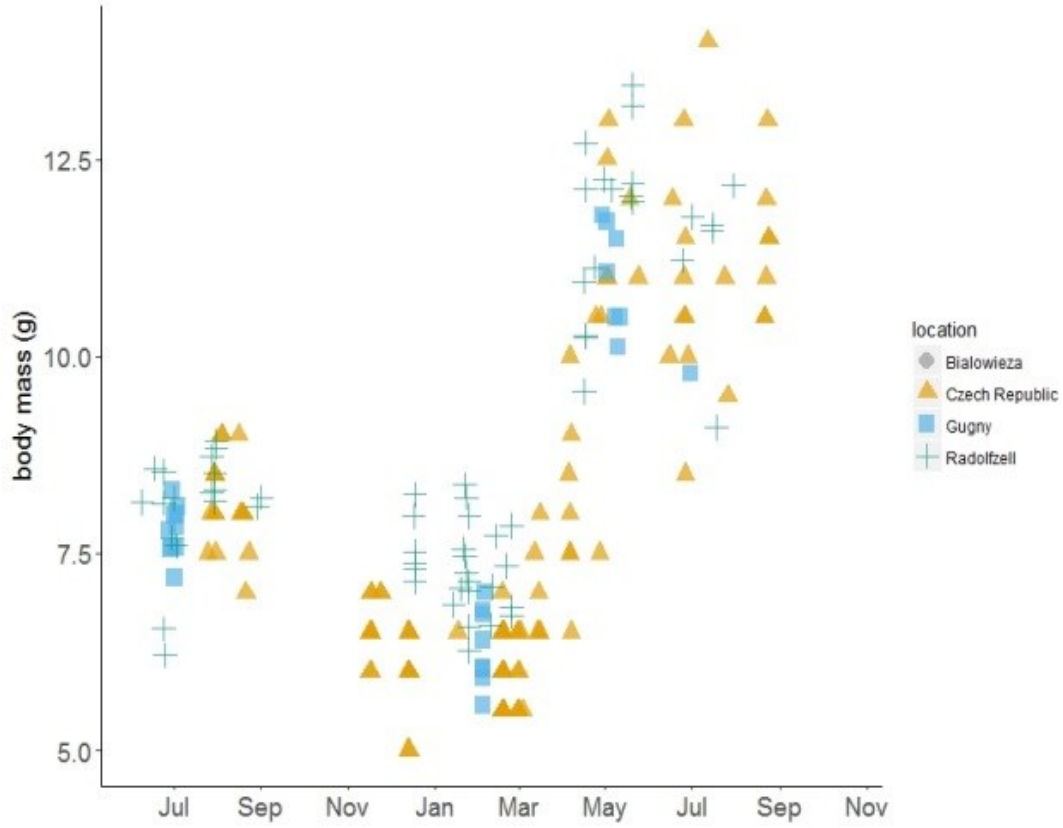
Figure 3. Seasonal variation in body mass in the four populations analyzed in detail.

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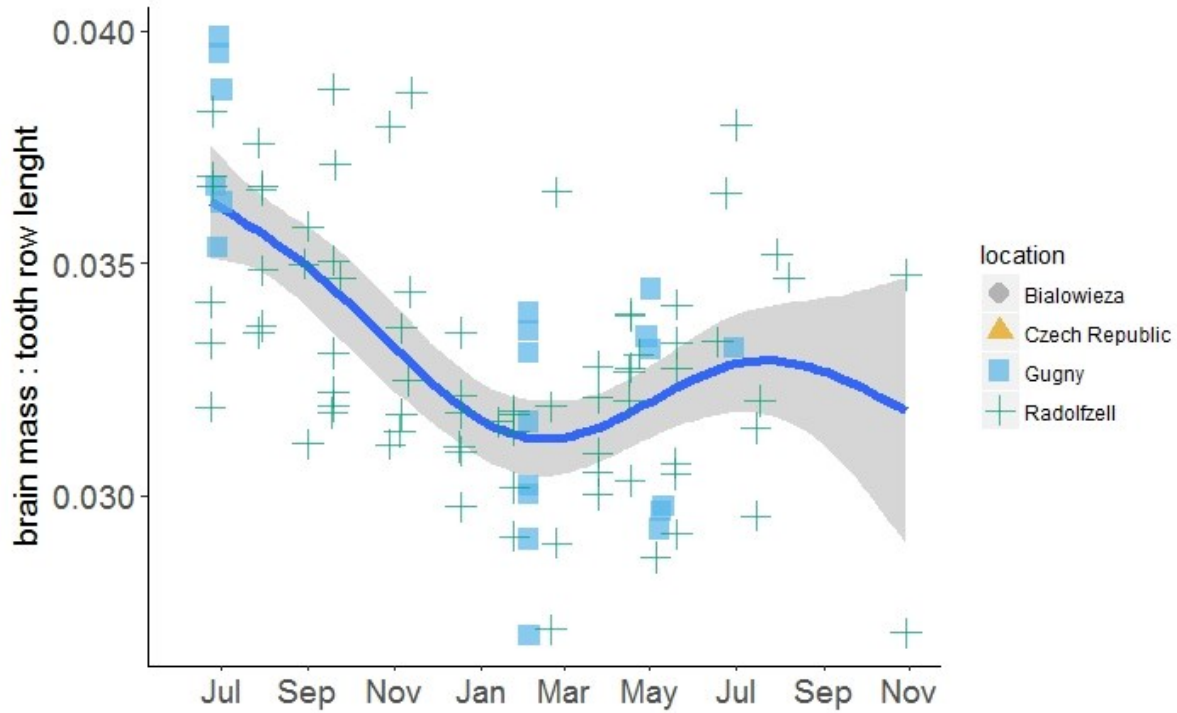
Figure 4. Seasonal variation in corrected brain mass in Gugny and Radolfzell with fitted Generalized Additive Model, using jday as smooth term (s), k=5. Solid line and shaded area represent fitted values and

standard error of the model, respectively (e.d.f.=3.67, F(s)=14, P(s)<0.001, deviance explained=38.4%). This fitted model helps to illustrate how adult Gugny brains were collected before the second size peak.

699
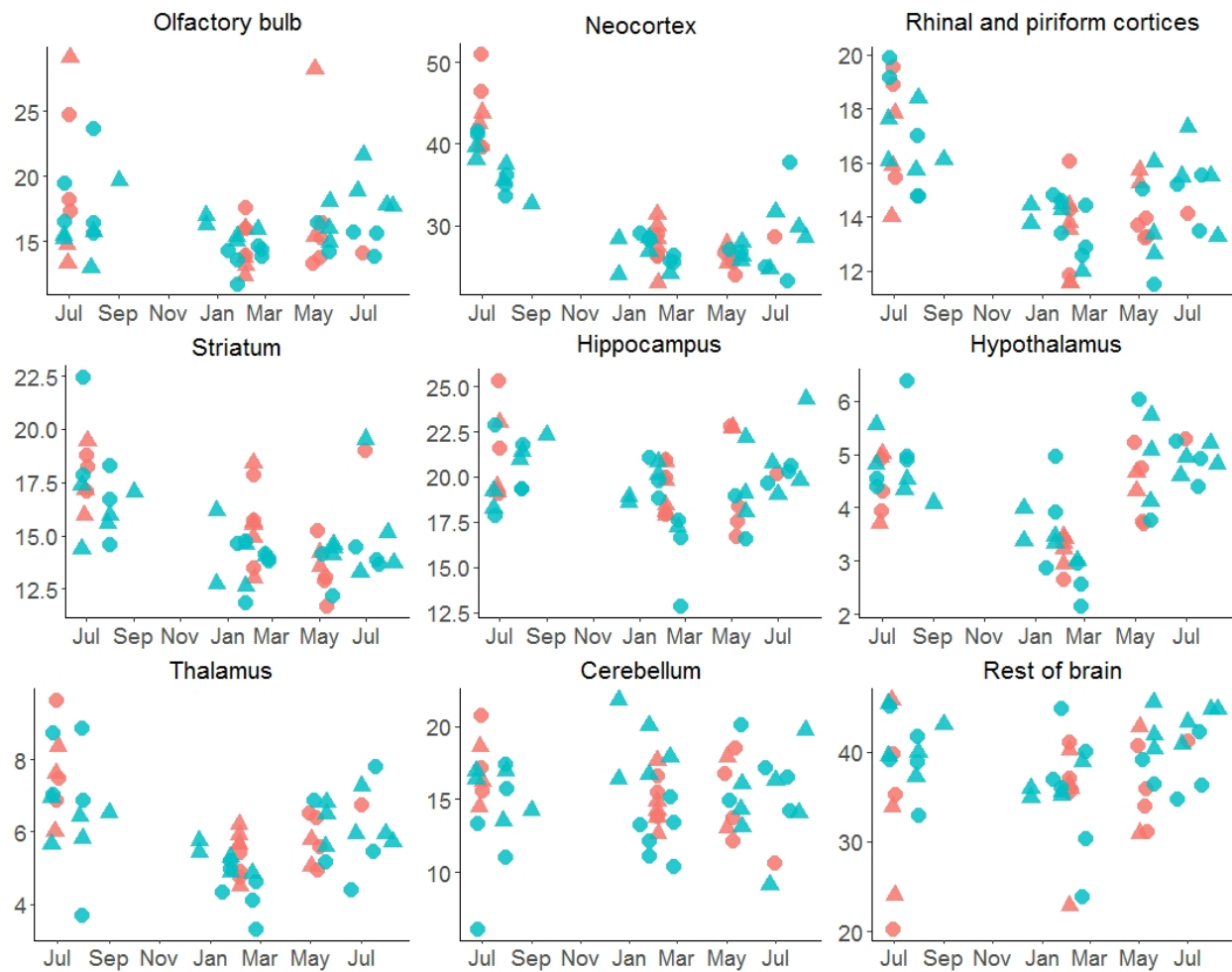
Figure 5. Variation between seasons and sexes in corrected volume of brain regions in Radolfzell (blue) and Gugny (red). As in Fig.4, the too early collection of adult brains in Gugny is evident.

705 **Table 1.** Results from linear models to test correlation between intensity in morphological changes

706 (DP) and geographical variables

Brain case height decrease					
	F	DF	Adj-R2	P	correlation
latitude	5.6	17	0.20	<0.05	positive
longitude	5.3	17	0.19	<0.05	positive
lat. X long.	6.6	17	0.24	<0.05	positive
altitude	0.0	15	0.01	>0.5	no
Brain case height regrowth					
latitude	0.1	14	0.03	>0.1	no
longitude	0.4	14	0.04	>0.5	no
lat. X long.	0.5	14	0.03	>0.1	no
altitude	2.2	12	0.09	>0.1	no
Brain mass decrease					
latitude	0.1	8	0.12	>0.5	no
longitude	0.2	8	0.10	>0.5	no
lat. X long.	0.2	8	0.10	>0.5	no
altitude	0.5	8	0.06	>0.1	no
Brain mass regrowth					
latitude	2.8	7	0.18	>0.1	no
longitude	0.7	7	0.03	>0.1	no
lat. X long.	0.7	7	0.04	>0.1	no
altitude	4.5	7	0.30	>0.05	no
Body mass decrease					
latitude	2.5	24	0.06	>0.1	no
longitude	10.8	24	0.28	<0.01	positive
lat. X long.	10.2	24	0.27	<0.01	positive
altitude	0.7	24	0.03	>0.1	no
Body mass regrowth					
latitude	0.4	18	0.03	>0.5	no
longitude	2.6	18	0.08	>0.1	no
lat. X long.	2.3	18	0.06	>0.1	no
altitude	0.9	18	0.01	>0.1	no

707

708　**Table 2.** Results from linear models testing correlation between intensity of morphological change
709　(DP) and climate variables.

Brain case height decrease					
	F	DF	Adj-R2	P	correlation
annual mean temperature	0.2	17	0.05	>0.5	no
mean diurnal temperature range	0.3	17	0.04	>0.5	no
isothermality	5.4	17	0.20	<0.05	negative
temperature seasonality	9.0	17	0.31	<0.01	positive
max. temperature of warmest month	0.9	17	0.01	>0.1	no
min. temperature of coldest month	3.1	17	0.11	>0.05	no
temperature annual range	9.8	17	0.33	<0.01	positive
mean temperature of wettest quarter	1.5	17	0.03	>0.1	no
mean temperature of driest quarter	24.8	17	0.57	>0.001	negative
mean temperature of warmest quarter	1.1	17	0.00	>0.1	no
mean temperature of coldest quarter	2.6	17	0.08	>0.1	no
annual precipitation	0.0	17	0.03	>0.1	no
precipitation of wettest month	0.0	17	0.06	>0.5	no
precipitation of driest month	1.5	17	0.03	>0.1	no
precipitation seasonality	7.6	17	0.27	<0.05	positive
precipitation of wettest quarter	2.3	17	0.06	>0.5	no
precipitation of driest quarter	2.1	17	0.06	>0.1	no
precipitation of warmest quarter	0.1	17	0.05	>0.5	no
precipitation of coldest quarter	2.9	17	0.10	>0.1	no
Brain case height regrowth					
annual mean temperature	2.9	14	0.06	>0.5	no
mean diurnal temperature range	0.2	14	0.06	>0.5	no
isothermality	0.2	14	0.05	>0.5	no
temperature seasonality	1.1	14	0.01	>0.1	no
max. temperature of warmest month	1.2	14	0.01	>0.1	no
min. temperature of coldest month	0.0	14	0.07	>0.5	no
temperature annual range	1.4	14	0.03	>0.1	no
mean temperature of wettest quarter	1.2	14	0.01	>0.1	no
mean temperature of driest quarter	1.8	14	0.05	>0.1	no
mean temperature of warmest quarter	1.0	14	0.00	>0.1	no
mean temperature of coldest quarter	0.0	14	0.07	>0.5	no
annual precipitation	1.6	14	0.04	>0.1	no
precipitation of wettest month	3.4	14	0.14	>0.05	no
precipitation of driest month	1.4	14	0.03	>0.1	no
precipitation seasonality	0.1	14	0.06	>0.5	no
precipitation of wettest quarter	3.5	14	0.14	>0.05	no
precipitation of driest quarter	0.9	14	0.01	>0.1	no
precipitation of warmest quarter	5.0	14	0.21	<0.05	positive
precipitation of coldest quarter	0.0	14	0.07	>0.5	no

Brain mass decrease					
annual mean temperature	0.1	8	0.11	>0.5	no
mean diurnal temperature range	0.2	8	0.09	>0.5	no
isothermality	0.0	8	0.12	>0.5	no
temperature seasonality	0.1	8	0.12	>0.5	no
max. temperature of warmest month	0.5	8	0.06	>0.5	no
min. temperature of coldest month	0.1	8	0.12	>0.5	no
temperature annual range	0.1	8	0.11	>0.5	no
mean temperature of wettest quarter	0.1	8	0.11	>0.5	no
mean temperature of driest quarter	0.1	8	0.12	>0.5	no
mean temperature of warmest quarter	0.1	8	0.11	>0.5	no
mean temperature of coldest quarter	0.1	8	0.12	>0.5	no
annual precipitation	0.1	8	0.12	>0.5	no
precipitation of wettest month	0.6	8	0.05	>0.1	no
precipitation of driest month	0.1	8	0.11	>0.5	no
precipitation seasonality	0.0	8	0.12	>0.5	no
precipitation of wettest quarter	0.2	8	0.10	>0.5	no
precipitation of driest quarter	0.0	8	0.12	>0.5	no
precipitation of warmest quarter	0.2	8	0.10	>0.5	no
precipitation of coldest quarter	0.1	8	0.11	>0.5	no
Brain mass regrowth					
annual mean temperature	1.1	7	0.02	>0.1	no
mean diurnal temperature range	0.1	7	0.13	>0.5	no
isothermality	1.7	7	0.08	>0.1	no
temperature seasonality	0.9	7	0.01	>0.1	no
max. temperature of warmest month	1.8	7	0.09	>0.1	no
min. temperature of coldest month	1.0	7	0.00	>0.1	no
temperature annual range	0.8	7	0.03	>0.1	no
mean temperature of wettest quarter	5.2	7	0.34	>0.05	no
mean temperature of driest quarter	1.2	7	0.03	>0.1	no
mean temperature of warmest quarter	5.2	7	0.34	>0.05	no
mean temperature of coldest quarter	1.0	7	0.00	>0.1	no
annual precipitation	3.0	7	0.20	>0.1	no
precipitation of wettest month	4.4	7	0.30	>0.05	no
precipitation of driest month	2.1	7	0.12	>0.1	no
precipitation seasonality	0.3	7	0.10	>0.5	no
precipitation of wettest quarter	4.1	7	0.28	>0.05	no
precipitation of driest quarter	2.3	7	0.14	>0.1	no
precipitation of warmest quarter	4.1	7	0.28	>0.05	no
precipitation of coldest quarter	2.0	7	0.11	>0.1	no
Body mass decrease					
annual mean temperature	6.6	24	0.18	<0.05	negative
mean diurnal temperature range	3.6	24	0.10	>0.05	no
isothermality	8.4	24	0.23	<0.01	negative
temperature seasonality	13.5	24	0.33	<0.01	positive
max. temperature of warmest month	0.8	24	0.01	>0.1	no

min. temperature of coldest month	10.5	24	0.28	<0.01	negative
temperature annual range	13.7	24	0.34	<0.01	positive
mean temperature of wettest quarter	6.1	24	0.17	<0.05	positive
mean temperature of driest quarter	16.3	24	0.38	>0.001	negative
mean temperature of warmest quarter	0.0	24	0.04	>0.5	no
mean temperature of coldest quarter	10.5	24	0.28	<0.01	negative
annual precipitation	4.0	24	0.11	>0.05	no
precipitation of wettest month	0.1	24	0.04	>0.5	no
precipitation of driest month	6.4	24	0.18	<0.05	negative
precipitation seasonality	9.0	24	0.24	<0.01	positive
precipitation of wettest quarter	0.4	24	0.03	>0.5	no
precipitation of driest quarter	7.9	24	0.22	<0.01	negative
precipitation of warmest quarter	0.8	24	0.01	>0.1	no
precipitation of coldest quarter	10.4	24	0.27	<0.01	negative
Body mass regrowth					
annual mean temperature	1.8	18	0.04	>0.1	no
mean diurnal temperature range	0.7	18	0.02	>0.1	no
isothermality	3.1	18	0.10	>0.05	no
temperature seasonality	2.9	18	0.09	>0.1	no
max. temperature of warmest month	0.2	18	0.04	>0.5	no
min. temperature of coldest month	2.2	18	0.06	>0.1	no
temperature annual range	2.4	18	0.07	>0.1	no
mean temperature of wettest quarter	2.1	18	0.05	>0.1	no
mean temperature of driest quarter	5.3	18	0.18	<0.05	negative
mean temperature of warmest quarter	0.0	18	0.06	>0.5	no
mean temperature of coldest quarter	2.4	18	0.07	>0.1	no
annual precipitation	0.1	18	0.05	>0.5	no
precipitation of wettest month	1.0	18	0.00	>0.1	no
precipitation of driest month	0.5	18	0.02	>0.1	no
precipitation seasonality	4.7	18	0.16	<0.05	positive
precipitation of wettest quarter	0.5	18	0.02	>0.1	no
precipitation of driest quarter	1.2	18	0.01	>0.1	no
precipitation of warmest quarter	2.9	18	0.09	>0.1	no
precipitation of coldest quarter	1.7	18	0.04	>0.1	no

710

711 **Table 3.** Summary of morphological changes between DP stages in the four studied populations.

	summer juvenile			winter subadult			spring/summer adult			summer - winter change	winter - adult change
BCH (mm)	n	mean	period	n	mean	period	n	mean	period		
Radolfzell	20	6.21	Jun-Jul	10	5.46	Feb	9	5.89	May-Jun	-12.1%	7.9%
Gugny	6	6.46	Jun-Jul	8	5.67	Feb	7	6.09	May-Jun	-12.2%	7.4%
Žofín	8	5.99	Jul	27	5.22	Feb	7	5.83	Aug	-12.9%	11.7%
Bialowieza	23	5.62	Jun	4	4.77	Jan-Feb	16	5.31	Jun	-15.1%	11.3%
Corr. brain mass (g/mm)											
Radolfzell	12	0.035	Jun-Jul	4	0.031	Feb	9	0.032	May-Jun	-11.4%	3.2%
Gugny	6	0.038	Jun-Jul	8	0.032	Feb	6	0.032	May-Jun	-15.8%	0.0%
Body mass (g)											
Radolfzell	8	8.37	Jul	7	7.15	Feb	7	12.49	May	-14.6%	74.7%
Gugny	10	7.79	Jun-Jul	8	6.31	Feb	6	10.9	May	-19.0%	72.7%
Žofín	10	8.15	Aug	26	6.02	Feb	7	11.43	Aug	-26.1%	89.9%

712

713 **Supplement material for:**

714 Seasonal reversible size changes in small high-metabolic mammals exceed geographic variation - a
715 review of Dehnel's Phenomenon in the common shrew

716

717 **Results on SKL and BCW variation in four populations of the common shrew**

718 Seasonal variation in SKL was less pronounced than in BCH (Table S1). The models comparison revealed
719 no significant effect of sex in SKL variation (AIC(M1)= 145.0, AIC(M2)=130.3, ANOVA; P>0.5). In the final
720 model M2 (d.f.=191, adj. R2=0.78, F(season)=44.3, F(location)=67.7, F(interaction seasons:location)=1.6),
721 there was a difference between seasons and locations at the factor level (P<0.001 both) but not their
722 interaction (P>0.1). We only found a significant decline from summer juveniles to winter subadults in
723 Radolfzell (Tukey test, P<0.001); we found no other differences between seasons in any other location.

724 Variation in BCW was also very low (Table S1). Differences in sex were not significant (AIC(M1)= 29.0,
725 AIC(M2)=22.2, ANOVA; P>0.1). The final mode M2 (d.f.=179, adj. R2=0.29, F(season)=14.9,
726 F(location)=19.0, F(interaction seasons:location)=0.8) showed difference between seasons and locations
727 at the factor level (P<0.001 both) but not their interaction (P>0.5). However, we only found a significant
728 increase from winter subadults to adults in Radolfzell (Tukey test, P<0.05).

729 **Table S1**. Summary of changes in SKL and BCW in the four studied populations.

	summer juvenile			winter subadult			spring/summer adult			summer - winter change	winter - adult change
SKL (mm)	n	mean	period	n	mean	period	n	mean	period		
Radolfzell	19	20.42	Jun-Jul	24	19.76	Feb	9	19.96	May-Jun	-3.2%	1.0%
Gugny	8	20.14	Jun-Jul	8	19.92	Feb	7	19.75	May-Jun	-1.0%	-0.8%
Žofín	9	19.34	Jul	25	18.99	Feb	7	19.20	Aug	-1.8%	1.1%
Bialowieza	40	19.77	Jun	4	19.38	Jan-Feb	16	19.42	Jun	-2.0%	0.2%
BCW (mm)											
Radolfzell	20	9.60	Jun-Jul	24	9.51	Feb	14	9.81	May-Jun	-0.9%	3.2%
Gugny	8	9.62	Jun-Jul	8	9.39	Feb	7	9.72	May-Jun	-2.4%	3.5%
Žofín	8	9.19	Jul	27	9.18	Feb	17	9.38	Aug	-0.1%	2.1%
Bialowieza	35	9.51	Jun	3	9.46	Jan-Feb	20	9.61	Jun	-0.5%	1.6%

730

731

Table S2. Compilation of studies reporting seasonal changes in skull and/or brain size in other species
different than S. araneus.

species	location	measurement	summer - winter change	winter - adult change	reference
Blarina brevicauda	Ithaca, US	skull height	-6.14	4.26	Dapson 1968
Clethrionomys rutilus	Pyshma River, Taliza, Russia	brain mass	-10.3	19.6	Yaskin 1984
Crocidura suaveolens	Askania Nova, Ukraine	skull height	-10.12	4.95	Mezhzherin 1988
Microtus gregalis	Pyshma River, Taliza, Russia	brain mass	-12.7	26.5	Yaskin 1984
Microtus oeconomus	Pyshma River, Taliza, Russia	brain mass	-10.8	26.7	Yaskin 1984
Mustela erminea	Several locations	skull height	-16.8(f) -14.3 (m)		LaPoint et al. 2017
Mustela nivalis	Several locations	skull height	-12.5(f) -7.3 (m)		LaPoint et al. 2017
Mustela nivalis	Bialowieza, Poland	skull height (corrected by CBL)	-15.5	8.3 (m)	Dechmann et al. 2017
Myodes glareolus	Pyshma River, Taliza, Russia	brain mass	-8.7	13	Yaskin 1984
Neomys fodiens	Poznan, Poland	skull height	-2.3		Kardynia & Rychlik 2011
Sorex arcticus	Yamal peninsula, Russia	skull height	-8.5		Pucek 1963
Sorex caecutiens	Former USSR (various locations)	skull height	-15.9		Pucek 1963
Sorex longirotris	Alabama and Georgia, US	skull height	-4.73		French 1980
Sorex minutus	Gugny, Poland	brain mass	-17	7.65	Bartkowska et al. 2008
Sorex minutus	Bialowieza, Poland	skull height	-14.79	14.56	Caboń 1956
Sorex minutus	Bialowieza, Poland	braincase capacity	-30.04	21.41	Caboń 1956
Sorex minutus	Bialowieza, Poland	brain mass	-34.29	20.35	Caboń 1956
Sorex minutus	Bialowieza, Poland	skull height	-18.11	11.78	Dehnel 1949
Sorex minutus	Puławy, Poland	skull height	-19.1	15.5	Kubik 1951
Sorex minutus	Pyshma River, Taliza, Russia	brain mass	-31.7	21	Yaskin 1984
Sorex unguiculatus	Former USSR	skull height	-13.7	11.4	Pucek 1963
Sorex vagrans	Maple Ridge, Vancouver, Canada	skull height	-8.7		Hawes 1969

735 **References**

736 The following references correspond to the citations in Table S2 as well as the review supplementary
737 data from which the DP change values were extracted for the statistical analyses.

738 Bartkowska K, Djavadian RL, Taylor JRE, Turlejski K (2008) Generation recruitment and death of brain cells
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820

Location	Reference	Latitude	Longitude	Altitude	Species	Metric	% decrease from t1 to t2	% increase from t2 to t3	nt1	nt2	nt3	t1	t2	t3	mean_t1	mean_t2	mean_t3
NE Poland	Bartkowska et al. 2008	53,348259	22,592762	106	*Sorex araneus*	brain mass	10	7,9	16	17	NA	Jun-Aug	Dec-Feb	Jun-Aug	0,212	0,194	NA
Bialowieza, Poland	Bielak & Pucek 1960	52,728783	23,870556	183	*Sorex araneus*	brain mass	29,56	15,39	38	11	22	Jul	Feb-Mar	Jul	0,213	0,153	0,176
Bialowieza, Poland	Bielak & Pucek 1960	52,728783	23,870556	183	*Sorex araneus*	braincase capac	26,52	15,73	NA	NA	NA	Jul	Feb	Jul	NA	NA	NA
Bialowieza, Poland	Bielak & Pucek 1960	52,728783	23,870556	183	*Sorex araneus*	skull height	12,69	10,34	NA	NA	NA	Jul	Feb	Jul	NA	NA	NA
Bialowieza, Poland	Dehnel 1949	52,728783	23,870556	183	*Sorex araneus*	body mass	24	102,6	NA	NA	NA	Sep	Jan	Aug	NA	5,7	11,55
Bialowieza, Poland	Dehnel 1949	52,728783	23,870556	183	*Sorex araneus*	skull height	17,67	12,83	22	10	24	Jun	Jan+Feb	Jun	6,34	5,22	5,89
Bialowieza, Poland	Dehnel 1949	52,728783	23,870556	183	*Sorex araneus*	skull length	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bialowieza, Poland	Dehnel 1949	52,728783	23,870556	183	*Sorex araneus*	skull width	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lednice, Czech Republic	Homolka 1980	48,799918	16,803398	173	*Sorex araneus*	skull height	12,4	14,7	NA	NA	NA	Jun-Jul	Feb	May	6,05	5,3	6,08
Lednice, Czech Republic	Homolka 1980	48,799918	16,803398	173	*Sorex araneus*	body mass	22,35	95,45	NA	NA	NA	Jun	Jan	May	8,25	6,6	12,9
Wroclaw, Poland	Kowalska-Dyrcz 1961	51,1166667	17,0833333	155	*Sorex araneus*	body mass	21,7	90,6	21	5	9	Jun	Jan+Feb	Jun	7,04	5,51	10,5
Wroclaw, Poland	Kowalska-Dyrcz 1961	51,1166667	17,0833333	155	*Sorex araneus*	brain mass	22,1	11,5	21	4	10	Jun	Jan+Feb	Jun	0,21	0,164	0,182
Wroclaw, Poland	Kowalska-Dyrcz 1961	51,1166667	17,0833333	155	*Sorex araneus*	skull height	14,4	8,9	24	4	10	Jun	Jan+Feb	Jun	6,33	5,42	5,9
Pulawy, Poland	Kubik 1951	51,41655	21,96939	115	*Sorex araneus*	skull height	13,7	7,2	63	5	8	Jul	Jan-Feb	Jul-Aug	5,7	5,42	5,81
Moggingen, Germany	Lázaro et al. 2017	47,766042	8,99703	419	*Sorex araneus*	body mass	17,6	83	21	9	9	Jul	Jan	May	8,29	6,78	12,8
Moggingen, Germany	Lázaro et al. 2018	47,766042	8,99703	419	*Sorex araneus*	brain mass	21,7	17,6	6	4	5	Jun	Feb	Jul	0,252	0,202	0,224
Moggingen, Germany	Lázaro et al. 2018	47,766042	8,99703	419	*Sorex araneus*	skull height	12,9	13,8	8	9	4	Jul	Feb	Jun-Aug	6,27	5,85	6,22
Zofin, Czech Republic	Present study	48,671838	14,690402	750	*Sorex araneus*	body mass	26,13	89,87	10	26	7	Aug	Feb	Aug	8,15	6,02	11,43
Gugny, Poland	Present study	53,348259	22,592762	106	*Sorex araneus*	body mass	19	72,74	10	8	6	Jun-Jul	Feb	May	7,79	6,31	10,9
Gugny, Poland	Present study	53,348259	22,592762	106	*Sorex araneus*	brain mass	14,3	NA	6	8	7	Jun+Jul	Feb	May-Aug	0,259	0,222	NA
Zofin, Czech Republic	Present study	48,671838	14,690402	750	*Sorex araneus*	skull height	12,9	11,7	8	27	7	Jul	Feb	Aug	5,99	5,22	5,83
Gugny, Poland	Present study	53,348259	22,592762	106	*Sorex araneus*	skull height	12,2	7,4	6	8	7	Jun+Jul	Feb	May+Jun	6,46	5,67	6,09
Bialowieza, Poland	Pucek 1955	52,728783	23,870556	183	*Sorex araneus*	body mass	21,87	92,52	9	18	14	Oct	Jan+Feb	Jul	7,36	5,75	11,07
Bialowieza, Poland	Pucek 1955	52,728783	23,870556	183	*Sorex araneus*	braincase capac	28,57	18,86	20	6	8	Jun	Feb	Jun	254,5	175,83	208,75
Bialowieza, Poland	Pucek 1955	52,728783	23,870556	183	*Sorex araneus*	skull height	15,02	12,03	19	6	12	Jun	Feb	Jun	6,26	5,32	5,96
Bialowieza, Poland	Pucek 1955	52,728783	23,870556	183	*Sorex araneus*	skull length	1,73	1,23	28	7	14	Jun	Feb	Jul	19,09	18,76	18,99
Bialowieza, Poland	Pucek 1955	52,728783	23,870556	183	*Sorex araneus*	skull width	2,23	3,01	21	14	12	Jun	Feb	Jul	9,86	9,64	9,93
Former USSR, various locations	Pucek 1963	NA	NA	NA	*Sorex araneus*	skull height	16,7	13,7	40	24	19	Jun	Feb	Jun	6,28	5,23	5,94
Moscow, Russia	Pucek 1963	55,752586	37,590879	156	*Sorex araneus*	skull height	12,5	NA	NA	NA	NA	Jun	Feb	NA	NA	NA	NA
Bulgaria and former Czechoslovakia	Pucek 1963	NA	NA	NA	*Sorex araneus*	skull height	9	7,3	14	5	6	Jun	Feb	Jun	NA	NA	NA
Former USSR, various locations	Pucek 1963	NA	NA	NA	*Sorex araneus*	skull width	1,8	2,7	151	118	79	Jun-Nov	Dec-Apr	May-Sep	9,62	9,45	9,71
Bialowieza, Poland	Pucek 1965b	52,728783	23,870556	183	*Sorex araneus*	body mass	32,1	106,7	70	32	62	Sep	Feb	Jun	NA	NA	NA
Bialowieza, Poland	Pucek 1965b	52,728783	23,870556	183	*Sorex araneus*	brain mass	21,2	6,8	142	97	124	Jun-Aug	Dec-Feb	May-Jul	0,256	0,21	0,22
Vitosha, Bulgaria	Pucek & Markov 1964	42,5663889	23,2833333	NA	*Sorex araneus*	skull height	9,9	7,08	29	28	35	Jul-Aug	Jan+Feb	Jul-Aug	5,96	5,37	5,75
Vitosha, Bulgaria	Pucek & Markov 1964	42,5663889	23,2833333	NA	*Sorex araneus*	skull length	NA	NA	NA	NA	NA	Jul-Aug	Jan+Feb	Jul-Aug	NA	NA	NA
Vitosha, Bulgaria	Pucek & Markov 1964	42,5663889	23,2833333	NA	*Sorex araneus*	skull width	2	2,76	48	17	63	Jun-Oct	Nov-Feb	Mar-Oct	9,58	9,41	9,67
Bialowieza, Poland	Pucek 1965a	52,728783	23,870556	183	*Sorex araneus*	brain mass	20,9	5,3	71	79	62	Jun-Aug	Jan-Mar	Jun-Aug	0,263	0,208	0,219
Berlin, Germany	Schubart 1958	52,5272222	13,3819444	36	*Sorex araneus*	body mass	28,92	96,61	43	14	14	Oct	Feb	Aug	8,3	5,9	11,6
Berlin, Germany	Schubart 1958	52,5272222	13,3819444	36	*Sorex araneus*	skull height	9,17	7,48	62	30	45	Jul	Feb	Jul	5,89	5,35	5,75
Berlin, Germany	Schubart 1958	52,5272222	13,3819444	36	*Sorex araneus*	skull length	0,59	1,62	60	29	17	Jul	Feb	Aug	18,63	18,52	18,82
Berlin, Germany	Schubart 1958	52,5272222	13,3819444	36	*Sorex araneus*	skull width	NA	3,04	60	23	44	Jul	Feb	Jul	9,17	9,2	9,48
Poznan, poland	Serafinski 1955	52,395	16,9333333	76	*Sorex araneus*	skull height	12,78	6,5	40	4	8	Jun	Dec-Jan	Jul	6,1	5,32	5,69
Helsinki, Finland	Skaren 1964	60,173333	24,948611	NA	*Sorex araneus*	skull height	14,31	10,06	13	6	25	Jun	Jan+Feb	Jun	6,15	5,27	5,8
Bialowieza, Poland	Taylor et al. 2013	52,728783	23,870556	183	*Sorex araneus*	body mass	17,8	NA	48	19	NA	Jun+Jul	Jan+Feb	NA	7,93	6,52	NA
Bialowieza, Poland	Taylor et al. 2013	52,728783	23,870556	183	*Sorex araneus*	body mass	18,7	NA	NA	NA	NA	Jun+Jul	Jan+Feb	NA	NA	NA	NA
Bialowieza, Poland	Taylor et al. 2013	52,728783	23,870556	183	*Sorex araneus*	body mass	20,5	NA	NA	NA	NA	Jun+Jul	Jan+Feb	NA	NA	NA	NA
Bialowieza, Poland	Taylor et al. 2013	52,728783	23,870556	183	*Sorex araneus*	skull height	12,2	NA	NA	NA	NA	Jun+Jul	Jan+Feb	NA	NA	NA	NA
Bialowieza, Poland	Taylor et al. 2013	52,728783	23,870556	183	*Sorex araneus*	skull height	14,1	NA	NA	NA	NA	Jun+Jul	Jan+Feb	NA	NA	NA	NA
Kalinin, Tver, Russia	Viktorov 1967	56,851976	35,933533	135	*Sorex araneus*	skull height	15,98	10,88	NA	NA	NA	Jun	Feb	NA	NA	NA	NA
Taliza, Russia, Russia	Yaskin 1994	57,033625	63,876613	123	*Sorex araneus*	body mass	24,5	73,92	103	18	62	Jun-Aug	Feb+Mar	Jun-Aug	7,06	5,33	9,27

Location	Source	Latitude	Longitude		Species	Trait											
Tuchkovo, Russia	Yaskin 1994	55,61588	36,458302	146	*Sorex araneus*	body mass	28,94	89,48	10	18	9	Jun-Aug	Feb+Mar	Jun-Aug	7,36	5,23	9,91
Taliza, Russia	Yaskin 1994	57,033625	63,876613	123	*Sorex araneus*	brain mass	19,77	6,76	103	18	62	Jun-Aug	Feb+Mar	Jun-Aug	0,258	0,207	0,221
Tuchkovo, Russia	Yaskin 1994	55,61588	36,458302	146	*Sorex araneus*	brain mass	26,33	7,25	10	18	9	Jun-Aug	Feb+Mar	Jun-Aug	0,262	0,193	0,207
Tuchkovo, Russia	Yaskin 1994	55,61588	36,458302	146	*Sorex araneus*	skull height	17,8	14,57	NA	NA	NA	Jun-Aug	Feb	Jun-Aug	6,01	4,94	5,36
Stockerau, Austria	Spitzenberger 2001	48,3846124	16,2076303	172	*Sorex araneus*	skull height	12	9	NA	NA	NA	NA	NA	NA	NA	NA	NA
Stockerau, Austria	Spitzenberger 2001	48,3846124	16,2076303	172	*Sorex araneus*	body mass	19,4	68,4	NA	NA	NA	NA	NA	NA	NA	NA	NA
The Hague, Netherlands	Croin Michielsen 1966	52,145891	4,368044	14	*Sorex araneus*	body mass	9,6	80,1	31	32	NA	Jul	Jan	NA	NA	NA	NA
The Hague, Netherlands	Croin Michielsen 1966	52,145891	4,368044	14	*Sorex araneus*	body mass	14,5	83,1	23	16	NA	Jul	Jan	NA	NA	NA	NA
Ascot, UK	Churchfield et al. 1995	51,4113329	-0,6428289	63	*Sorex araneus*	body mass	19,2	NA	NA	NA	NA	Oct	Feb	NA	NA	NA	NA
Oulu, Finland	Hyvarinen & Heikura 1971	65,017294	25,595138	15	*Sorex araneus*	body mass	26,9	NA	44	30	NA	Sep	Jan	NA	7,1	5,19	NA
Oulu, Finland	Hyvarinen 1969	65,017294	25,595138	15	*Sorex araneus*	body mass	24,6	88,61	13	29	11	Aug	Mar	Jun	7,07	5,33	10,05
Farnharm, UK	Churchfield 1981	51,1881125	-0,8441448	119	*Sorex araneus*	body mass	6,6	46	NA	NA	NA	NA	NA	NA	6,46	6,03	8,93
Monks Wood, UK	Churchfield 1981	52,405456	-0,2408409	24	*Sorex araneus*	body mass	11,5	33,6	NA	NA	NA	NA	NA	NA	7,13	6,31	9,5
Joensuu, Finland	Hyvarinen 1994	62,610277	29,771098	117	*Sorex araneus*	body mass	17,9	NA	16	11	NA	Aug+Sep	Jan+Feb	NA	6,7	5,5	NA
Oulu, Finland	Pasanen 1971	65,017294	25,595138	15	*Sorex araneus*	body mass	24,1	89,7	65	51	53	Sep	Jan	Jun	7,05	5,35	10,15
Frankfurt (Oder), Germany	Stein 1938	52,317133	14,569189	73	*Sorex araneus*	body mass	22,9	62,8	41	17	27	Oct	Feb	Apr	8,31	6,41	10,44
Estonia, various locations	Kuuse 1987	59,0560933	24,642334	NA	*Sorex araneus*	body mass	29,3	91,3	NA	NA	NA	Oct	Jan	Apr	8,44	5,97	11,38
Taliza, Russia	Yaskin 1984	57,033625	63,876613	123	*Sorex araneus*	brain mass	23,6	11,8	NA	NA	NA	Jun-Aug	Feb+Mar	Jun-Aug	NA	NA	NA

IV.   **Nováková Lucie** & Vladimír Vohralík (2019): Discrimination of the sympatric species of water shrews *Neomys fodiens* and *N. milleri* (Soricomorpha, Soricidae). Zoologischer Anzeiger, 283: 27 – 32.

Research paper

Discrimination of the sympatric species of water shrews *Neomys fodiens* and *N. milleri* (Soricomorpha, Soricidae)

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ABSTRACT

The water shrew species *Neomys fodiens* and *Neomys milleri* are morphologically similar and, based on phenotype, it is difficult to distinguish them. Mandibles of both species were studied by means of classic as well as geometric morphometrics. We compared *a priori* identified mandibles of water shrews captured in two areas where the species ranges partially or entirely overlap, i.e., in Germany and in the south-eastern part of the Balkan Peninsula. Discrimination of the two species by linear measurements was successful in Germany but not in the Balkans. Discriminant analysis revealed clear differences in the shape of the mandible between the species in both regions. Comparison of allopatric and sympatric populations of both species showed that species are smaller and more similar in shape where they occur in sympatry. Our results suggest that geometric morphometrics can be successfully used for species determination, especially in regions where determination by size is not possible.

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1. Introduction

A sympatric and syntopic occurrence of two closely related species is usually associated with similar adaptations, resulting in a similar phenotype; such species are denoted as sibling or cryptic species (Mayr 1963). This is the case in European water shrews of the genus *Neomys*. There is an extensive overlap of the areas of distributions in two of them (Fig. 1), *Neomys fodiens* (Pennant, 1771) and *Neomys milleri* Mottaz, 1907. *Neomys milleri* was for a long time considered as subspecies of *N. anomalus* Cabrera, 1907 and recently elevated to the species level (Igea et al. 2015; Querejeta & Castresana 2018). The distributional areas of *Neomys* species differ considerably; *N. fodiens* inhabits a vast area between Great Britain and Japan, while *N. milleri* is confined to the region between the Pyrenees Mountains and the west of the European Russia. These two *Neomys* species can be distinguished by the shape of the glans penis or a combination of several body and cranial measurements (Pucek 1964; Spitzenberger 1990). However, discrimination based solely on the skull is difficult, especially in the population of the Balkan Peninsula (Vohralík & Sofianidou 1987; Kryštufek & Griffiths 2000). Moreover, previous studies focused mostly on

size differences (e.g., Ruprecht 1971; Spitzenberger 1980; Kryštufek & Griffiths 2000), with only two studies examining the differences in skull shape (Rácz & Demeter 1998; Rychlik et al. 2006).

Since shrews rarely adopt a semi-aquatic way of life (Hutterer 1985), information about possible adaptations of water shrews to various environmental factors and about the effect of competition is scarce. Some studies suggest that environment and habitat characteristics play a more important role in phenotypic adaptations than species competition (Rychlik et al. 2006; Popov & Zidarova 2008). Changes in body and skull size along the altitudinal gradient were also observed in *Neomys* species (Spitzenberger, 1980). The general pattern of morphological changes in water shrews with gradients of various biotic and environmental factors still remains unclear.

The aim of our study was to discriminate between two *Neomys* species using geometric morphometrics in *a priori* identified specimens collected in Bulgaria and Greece, i.e., in regions where species identification is challenging. For comparison, we used *a priori* identified specimens from Germany where morphological differences between the two species are more pronounced (Rempe & Bühler 1969; Ruprecht 1971). We also tested the influence of allopatry and sympatry on morphological traits of *Neomys* water shrews.
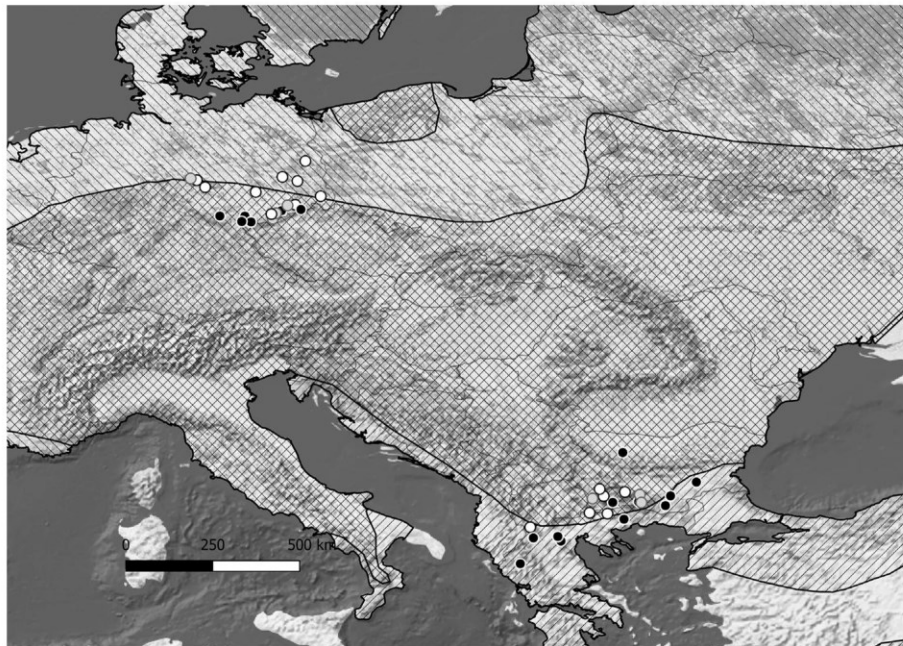
Fig. 1. Map of distribution of the *Neomys* species. *N. fodiens*, //*N. milleri*. Trapping locations of the studied specimens: *N. fodiens* – white dots, *N. milleri* – black dots, both species – grey dots.

2. Material and methods

2.1. Specimens

We evaluated 173 individuals of the Eurasian water shrew (*N. fodiens*) and the Miller's water shrew (*N. milleri*). The water shrews were captured in two areas where the species ranges partially or entirely overlap, in Germany and in the south-eastern part of the Balkan Peninsula (Fig. 1). The material from Germany consisted of 74 specimens of *N. fodiens* and 25 specimens of *N. milleri* caught between 1949 and 1990, which are deposited in the Museum für Naturkunde Berlin (MfN), Germany (Supplementary material, Table S1). The material from the Balkans consisted of 39 specimens of *N. fodiens* and 35 specimens of *N. milleri* captured by the second author in Bulgaria and Greece between 1972 and 1990. The specimens are deposited in the collections of the Department of Zoology at the Charles University, Czech Republic (Supplementary material, Table S2).

We assumed that the specimens from the MfN had been correctly assigned to species, since in Germany this can be done reliably using body measurements (cf. Spitzenberger 1990), which were available for most of the specimens. Conversely, in the Balkan Peninsula, correct species determination is difficult (Kryštufek & Petkovski 1989; Kryštufek & Griffiths 2000). It is necessary to include several cranial, mandibular and body measurements or a detailed morphological analysis of the entire specimen (Pucek 1964; Kryštufek et al. 2000). Therefore, in our study we included only those Balkan specimens that were previously determined based on a combination of several skull and body measurements by Vohralík (1985) and Vohralík & Sofianidou (1987, 1992).

We included all available specimens regardless of trapping date or age to make sure that our measurements represented the standard population structure under natural conditions. Although the age variation in mandible measurements is only small (Spitzenberger 1980; Spitzenberger & Bauer 2001a, 2001b), we tested for age differences in our Balkan material. Spitzenberger (1980) and Spitzenberger & Bauer (2001a, 2001b) found sexual dimorphism in some mandible measurements. We tested for sex differences only in specimens from the Balkans, as in these specimens sex had been determined during dissection. We divided the Balkan material into non-overwintered individuals born in the year in which they were trapped (juveniles) and overwintered individuals (adults), based on the date of trapping and teeth abrasion, and into males and females, resulting in four groups. The shape and size differences between age classes and sexes were not significant. Therefore, we pooled data from all sex and age groups for further analyses (for details, see Supplementary material, Fig. S1).

We compared allopatric and sympatric populations of *N. fodiens* in the German material and *N. milleri* in the Balkan material. Sympatric and allopatric *N. fodiens* populations from Germany were compared with German *N. milleri*, which in Germany occurs only in sympatry with *N. fodiens*. Similarly, sympatric and allopatric populations of *N. milleri* from the Balkans were compared with *N. fodiens* from the Balkans, which occurs only in sympatry with *N. milleri* in this part of its area of distribution. We considered the populations of the species allopatric if they occurred outside the area of distribution of the second species according IUCN maps of distribution. We identified sympatric populations as those where we have proof of occurrence of both species. Therefore, we included into this category shrews only from locations where both species were trapped (Fig. 1, grey dots). The dataset for testing character displacement consisted of allopatric *N. milleri* (n = 11), sympatric *N. milleri* (n = 9), allopatric *N. fodiens* (n = 22) and sympatric *N. fodiens* (n = 44).

2.2. Morphometrics

We only measured perfectly preserved and intact left mandibles. The buccal side of each hemimandible was photographed with a high-resolution camera (Canon EOS 70D with Canon EF-S60 mm f/2.8 Macro USM lens) with an objective scale placed in every picture. We processed pictures of mandibles using the tps series software programs tpsUtil and tpsDig2 (Rohlf 2015). To assess size differences, we took two standard mammalogical measurements

(±0.1 mm accuracy), the length of mandible (LM) and the height of *processus coronoideus* (= coronoid process = height of mandible, HM; Fig. 2). We then placed 17 landmarks on the picture of each mandible to assess size and shape differences (Fig. 2, Table 1). All pictures and measurements were taken by the same person (L. N.).

We established measurement error in a subset of 35 individuals (ca. 20% of our specimens) by taking two pictures of every mandible and placing landmarks on each picture twice. We used Procrustes ANOVA (performed in MorphoJ; Klingenberg 2011) to test the effect of photographing and of landmark placing. The effect of measurement error was 22.5 times lower than the effect of an individual (the weakest biological signal in our dataset). Thus, we consider measurement error negligible and placed landmarks only once on each picture of a mandible.

2.3. Statistics

The coordinates of landmarks were superimposed by Generalized Procrustes Analysis (GPA) using MorphoJ (Klingenberg 2011). This step removed redundant information about position, rotation and size of the mandibles (Zelditch et al. 2012). The information about size of each mandible was preserved in the variable "centroid size" (CS). Centroid size is the square root of the sum of the squared distances of landmarks from their centroid (Zelditch et al. 2012). Procrustes coordinates (coordinates of landmarks after GPA) were used for further analyses.

First, we performed dataset exploration with principal component analysis (PCA). Then we applied a canonical variate analysis (CVA) with four groups of individuals (*N. fodiens* and *N. milleri* separately from the Balkans and from Germany) to highlight the differences between these groups. Additionally, we visualized shape changes of mandibles on the first CV axis as warped outline drawings. We then assigned individuals to species by discriminant function analysis (DF) with leave-one-out cross-validation for each study area. PCA, CVA and DF were performed in PAST v. 3.23 (Hammer et al. 2001).

To explore how allopatric and sympatric populations of both species differ in size, we used two-way analysis of variance (ANOVA) with centroid size as the dependent variable and species, allopatry/sympatry and their interaction as factors.

3. Results

3.1. Size differences

We took two linear measurements for each of the 173 studied individuals (Table 2). The two *Neomys* species overlap considerably
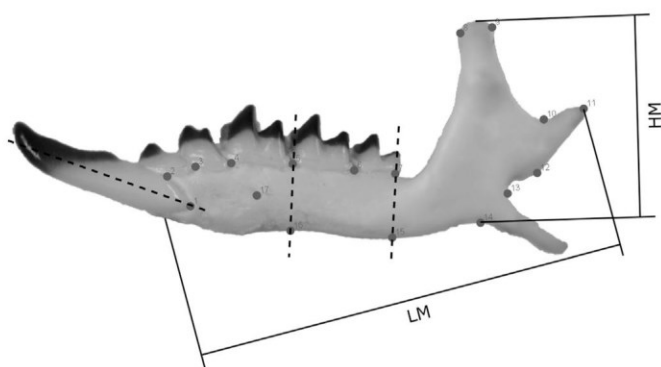
in the Balkans but are clearly separated in Germany (Fig. 3A). The linear measurements had the same range in both areas for *N. fodiens*, but there is a considerable shift in size of *N. milleri* over the geographic range.

The overall size of the species from each area, represented as centroid size, is shown in Fig. 3B. Centroid sizes of *N. milleri* from Germany were much smaller than CS of *N. milleri* from the Balkans and of *N. fodiens* from both study areas. Two-way ANOVA showed that sympatric populations of *N. milleri* as well as *N. fodiens* were significantly smaller ($F_{1,\ 82} = 9.627$, $p = 0.00263$) than allopatric populations in both species. Effect of species was also significant ($F_{1,\ 82} = 11.95$, $p = 0.00087$), while the interaction was not ($F_{1,\ 82} = 1.034$, $p = 0.3123$).

3.2. Shape differences

We visualized the shape data of mandibles after Procrustes superimposition using PCA. The distribution pattern defined by the first two axes (PC1 and PC2) explained 24.67% and 13.16% of the variation in our sample, respectively (Fig. 4). We used CVA to highlight the differences between species at our two study areas. The distribution pattern and shape changes of the mandible on the two axes are shown in Fig. 5. CVA completely distinguished almost all samples, with the only outlier being one *N. milleri* from the Balkans. The main differences in shape were in the size of *processus coronoideus* and the angle to *ramus mandibulae*. *Neomys fodiens* had a more robust mandible than *N. milleri* (Fig. 5).

Finally, the discriminant function distinguished mandibles of the two *Neomys* species from the Balkan Peninsula in all cases. Cross-validation correctly assigned 68 of the 74 evaluated specimens (i.e., 91.89%). Similarly, the discriminant function correctly identified 100% of the mandibles from Germany. In the ensuing cross-validation, only one of the 99 specimens was misidentified (i.e., 98.99% was identified correctly). See the supplementary material for frequency diagrams of the discriminant functions and of cross-validations (Fig. S2).

We compared allopatric and sympatric populations of both species, *N. fodiens* in Germany and *N. milleri* in the Balkans. Procrustes distances between species were larger in allopatric populations of *N. milleri* compared with allopatric *N. fodiens* than when the species occurred sympatrically. It means that the shape of mandible was more similar in above species when they occur sympatrically in comparison with situation in allopatric populations.

4. Discussion

We successfully distinguished mandibles of the two *Neomys* species from Germany by means of linear measurements as well as by means of geometric morphometrics. It was not possible to determine mandibles from the Balkan Peninsula based on linear measurements (cf. Table 2, Fig. 3A), but the species could be distinguished by geometric morphometrics. Results of both methods used showed that size and shape differences between mandibles from the evaluated areas are more pronounced in *N. milleri* in comparison to *N. fodiens*. Consequently, in our material, *N. milleri* showed higher phenotypic plasticity than *N. fodiens*. Correspondingly, Rychlik et al. (2006), who studied Polish water shrews, revealed greater differences in skull and mandible shape in populations of *N. milleri* than in *N. fodiens* populations. We suggest two explanations for these differences.

The first possible explanation of greater phenotypic plasticity in *N. milleri* is competition with *N. fodiens*. *Neomys fodiens* is competitively stronger than *N. milleri* and in sympatry, *N. milleri* is forced to phenotypically adapt and shift to a different ecological niche (Niethammer 1977, 1978). We can find similar or even more

Fig. 2. Mandible with position of 17 digitized landmarks and scheme of taking of two linear measurements.

Table 1
Description of positions of landmarks.

Landmark	Description of position
1	See Fig. 2 for definition
2	contact point of I_1, I_2 and the bone
3	contact point of I_2, P_4 and the bone
4	contact point of P_4, M_1 and the bone
5	contact point of M_1, M_2 and the bone
6	contact point of M_2, M_3 and the bone
7	posterior edge of tooth-row
8	anterior tip of the coronoid process
9	posterior tip of the coronoid process
10	maximum curvature on the curve between coronoid and articular processes
11	upper tip of the articular process
12	lower tip of the articular process
13	maximum curvature on the curve between articular and angular processes
14	maximum curvature on the curve between angular process and *corpus mandibulae*
15	See Fig. 2 for definition
16	See Fig. 2 for definition
17	*foramen mentale*

Table 2
Linear measurements in millimetres. Abbreviations: LM — length of mandible; HP — height of mandible; n — number of specimens; min — minimum value; max — maximum value; SE — standard error.

		n	mean	min	max	SE
LM						
Germany	*milleri*	25	9.70	9.2	10.2	0.0546
	fodiens	75	10.63	9.8	11.3	0.0433
Balkans	*milleri*	35	10.41	10.0	11.0	0.0420
	fodiens	39	10.36	9.5	11.3	0.0561
HP						
Germany	*milleri*	25	4.12	3.9	4.4	0.0247
	fodiens	75	4.94	4.5	5.4	0.0212
Balkans	*milleri*	35	4.58	4.2	4.9	0.0254
	fodiens	39	4.86	4.5	5.2	0.0262
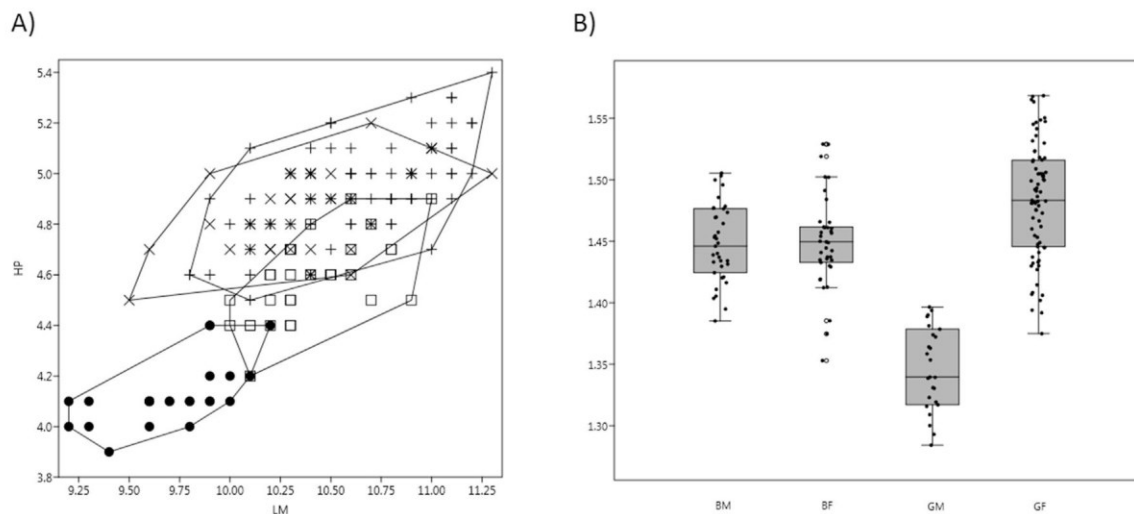
Fig. 3. A) Scatter diagram of the height of mandible (HM) and length of mandible (LM) in mm. Legends: □ − *N. milleri* from the Balkans, ● - *N. milleri* from Germany, × − *N. fodiens* from the Balkans, + − *N. fodiens* from Germany. B) Boxplots of centroid size, BM - *N. milleri* from the Balkans, GM - *N. milleri* from Germany, BF − *N. fodiens* from the Balkans, GF − *N. fodiens* from Germany.

pronounced examples in some other European small mammals, e.g., moles (*Talpa* spp.). These species exhibit only a small geographical overlap where one dominant species occupies an extensive area of distribution and the other, subordinate species, occupy much smaller areas (cf. Mitchell-Jones et al. 1999). For example, the European mole (*T. europaea*) is dominant throughout Europe, whereas only the south of the continent is populated by other *Talpa* species. Even habitats suitable for the other *Talpa* species according to ecological models are not inhabited by those species when *T. europaea* is present (Suárez-Seoane et al. 2014; Loy et al. 2017). The authors explain the situation by indirect competition. After the last glacial period, a competitively dominant species populated a large area in Central and Northern Europe, preventing other related species from spreading there.

Assuming the above described competitive scenario, we would expect character displacement to occur in sympatric populations.
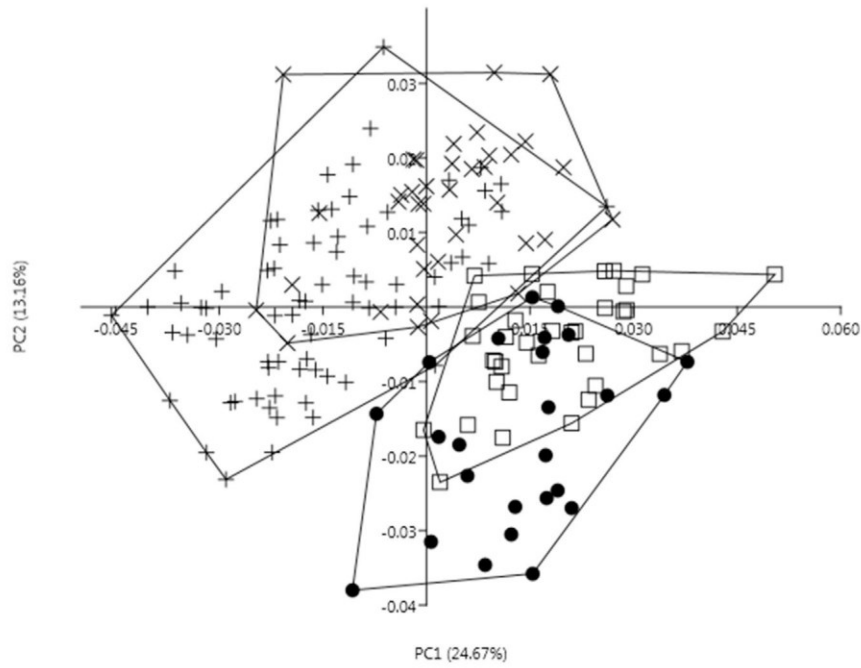
Fig. 4. Scatter plot of PCA of shape variables (Procrustes coordinates). Symbols are the same as in Fig. 3.
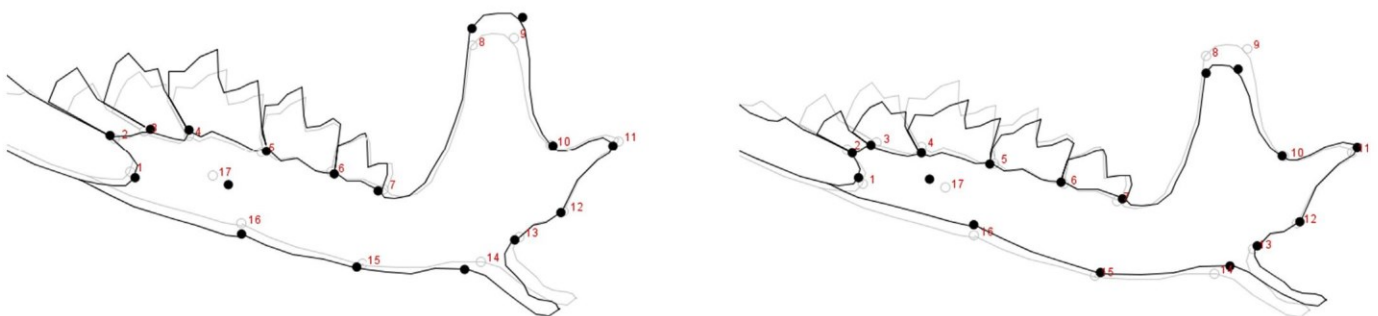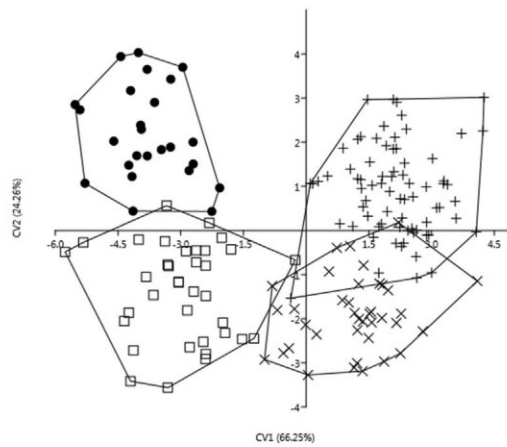

Fig. 5. Scatter plot of CVA of shape variables (Procrustes coordinates). Symbols are the same as in Fig. 3. Shape changes on a mandible along the first CV axes visualized as warped outline drawings.

Character displacement was tested in Polish *N. fodiens* (Rychlik et al., 2006). The area of distribution of water shrews in Poland does not allow to test character displacement in *N. milleri*. In our material, we tested character displacement in both species. Rychlik et al. (2006) did not find any differences between sympatric and allopatric populations of *N. fodiens*. Moreover, *N. fodiens* and *N. milleri* were more similar when they occurred in sympatry than in allopatry. This shows that environmental factors are more

important for adaptations of the species than competition (Rychlik et al. 2006). Our results based on more numerous materials agree with the findings of Rychlik et al. (2006) for *N. fodiens*. We also compared allopatric and sympatric populations of *N. milleri* and we did not find any differences or character displacement either.

The other plausible explanation for greater phenotypic plasticity in *N. milleri* is that *N. milleri* has a broader feeding spectrum than *N. fodiens*, with prevalence of terrestrial invertebrates (Kuviková 1987; Churchfield & Rychlik 2006). This higher dietary richness allows the species to venture into habitats farther away from water (Spitzenberger 1980) and adapt to various environmental conditions. Despite the described ecological advantage of *N. milleri*, *N. fodiens* has a much larger area of distribution (Hutterer et al. 2016). This discrepancy can be explained by the fact that *N. milleri* avoids the cold continental climate, so species competition is not the defining factor. The area of distribution of *N. milleri* is confined to warmer regions. In Central Europe, it is usually found between 200 and 600 m a.s.l. (Erfurt & Stubbe 1986; Baláž & Ambros 2007; Anděra 2010), occurrences at higher elevations are rare. Conversely, *N. fodiens* is better adapted to aquatic environment and is more cold-resistant (Spitzenberger 1980), colonizing much colder areas, including taiga and tundra.

When both species occur sympatrically and syntopically, they experience the same environmental conditions. We found that in such cases their phenotypes become more similar to each other and both species are smaller in comparison with their allopatric populations. Based on our results, we cannot distinguish which of the two factors (competition or environmental conditions) is crucial for shaping the phenotype of the water shrews.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2019.08.004.

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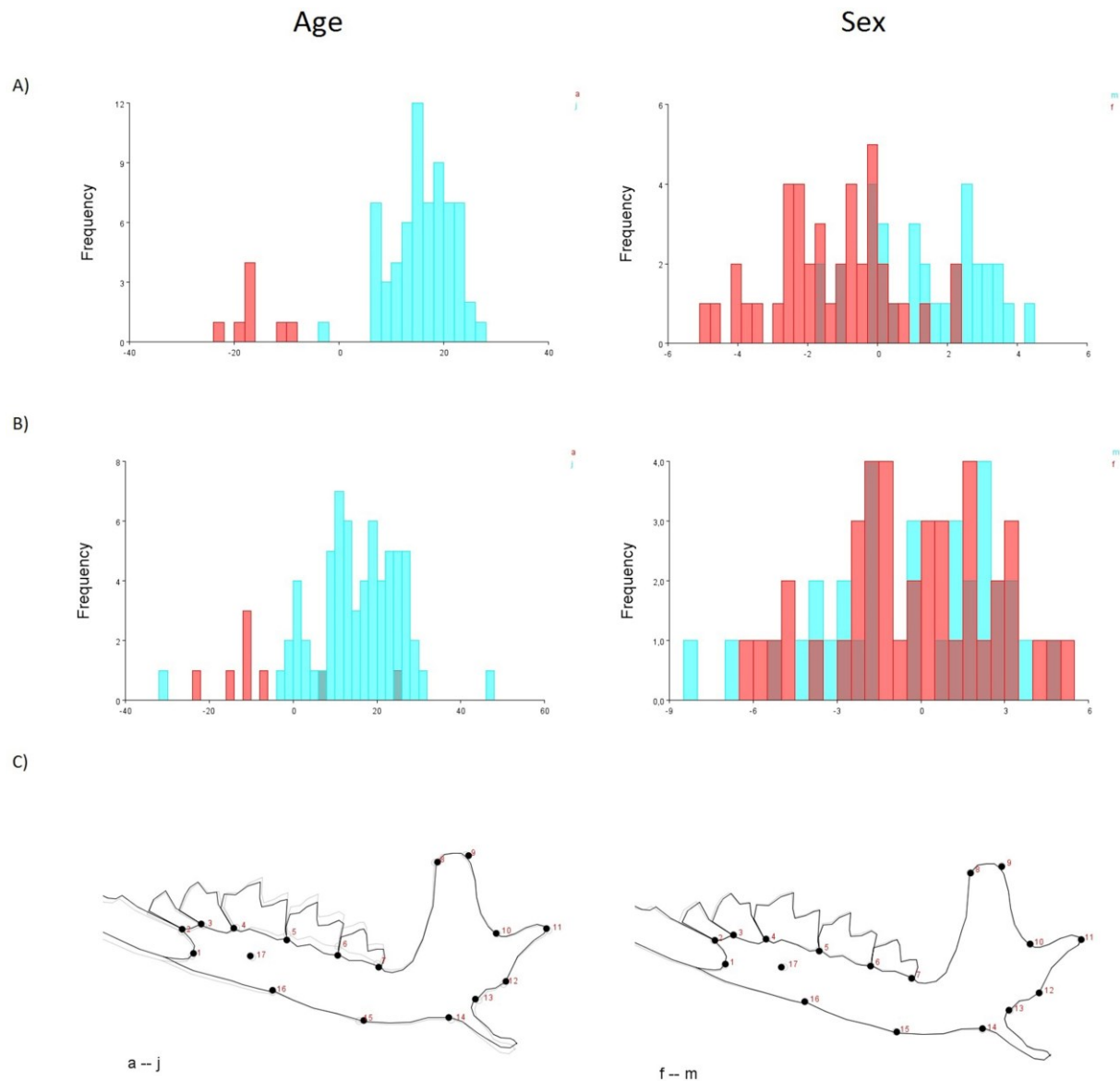
Fig. S1. Age and sex differences in *Neomys* from the Balkans. A) Frequency diagrams of the discriminant functions. Age: red – adults, blue – juveniles; sex: red – females, blue – males; B) frequency diagrams of cross-validations (explanations same as above); C) shape differences between age (left) and sex (right) groups visualised as warped outline drawings. Age: black – juveniles, grey – adults; sex: black – males, grey – females.
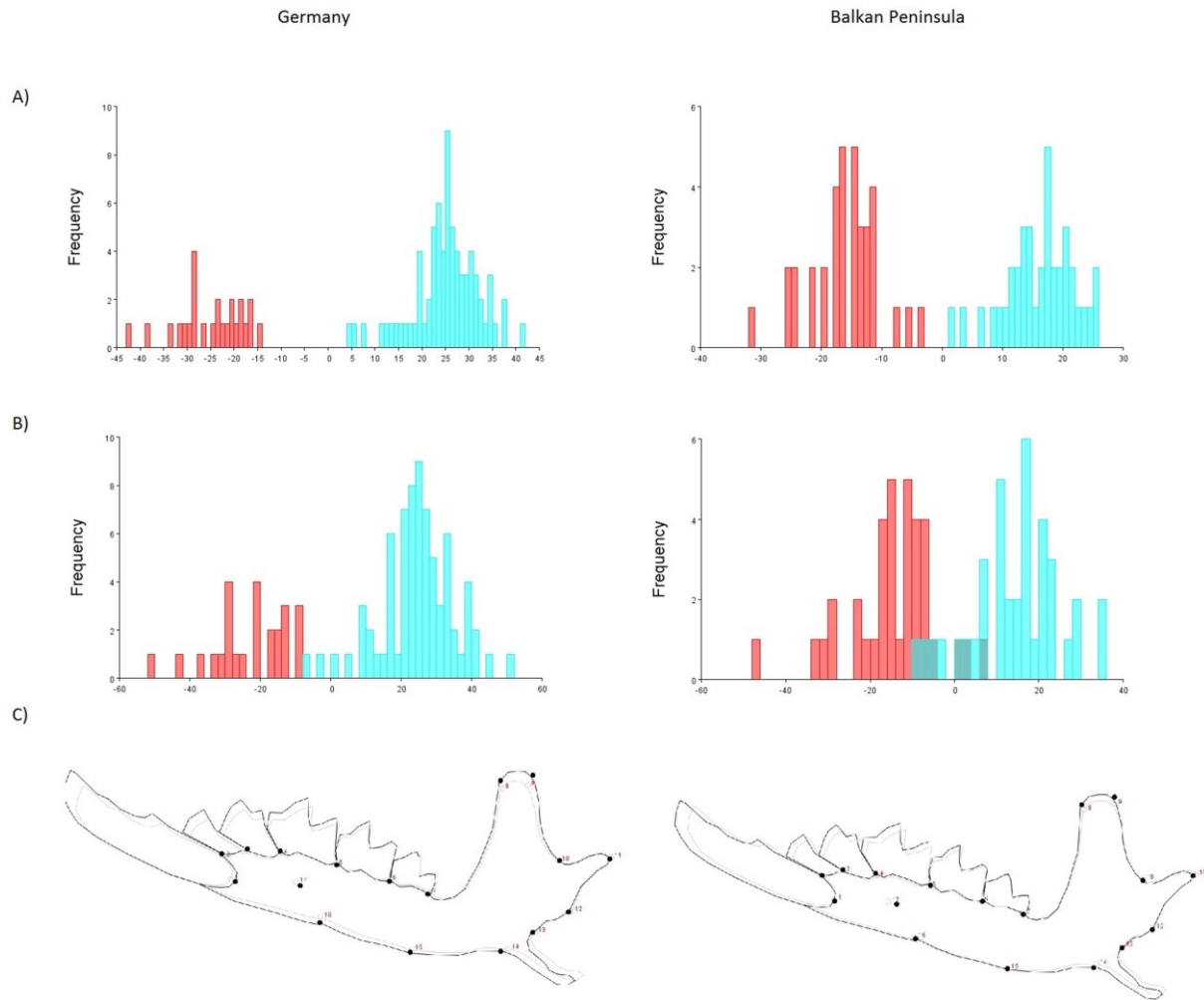
Fig. S2. Differences between *N. milleri* and *N. fodiens* for each study area. A) Frequency diagrams of the discriminant functions (red – *N. milleri,* blue – *N. fodiens*); B) frequency diagrams of cross-validations (explanations same as above); C) shape differences between species visualised as warped outline drawings (grey – *N. milleri*, black – *N. fodiens*).