The Great Lakes Entomologist

Volume 48
Numbers 3/4 -- Fall/Winter 2015 *Numbers 3/4 -- Fall/Winter 2015*
Article 1

October 2015

European Springtails Orchesella cincta (L.) and O. villosa (L.) (Collembola: Entomobryidae): Vagabond Species of the Nearctic Region

Catherine L. Smith
University of Tennessee

John K. Moulton
University of Tennessee, jmoulton@utk.edu

Ernest C. Bernard
University of Tennessee

Recommended Citation

Smith, Catherine L.; Moulton, John K.; and Bernard, Ernest C. 2015. "European Springtails Orchesella cincta (L.) and O. villosa (L.) (Collembola: Entomobryidae): Vagabond Species of the Nearctic Region," *The Great Lakes Entomologist*, vol 48 (3)
DOI: https://doi.org/10.22543/0090-0222.1014
Available at: https://scholar.valpo.edu/tgle/vol48/iss3/1

European Springtails *Orchesella cincta* (L.) and *O. villosa* (L.) (Collembola: Entomobryidae): Vagabond Species of the Nearctic Region

Catherine L. Smith[1], John K. Moulton[1*], and Ernest C. Bernard[1]

Abstract

North American specimens of the European invasive springtail *Orchesella cincta* (L.) were compared to several published European haplotypes in a phylogenetic framework using likelihood methods based on a portion of cytochrome oxidase II (*cox2*). Our analyses provide direct evidence of at least two distinct introductions of this invasive to North America from different regions of Europe. Additional introduction events cannot be ruled out because detection is limited by extremely low sequence divergence among populations inhabiting different regions of the continent. *Orchesella villosa* (L.), another invasive from Europe, is another candidate for multiple introductions. Herein we include the *cox2* sequence from single specimens of *O. villosa* from Maine and Oregon. Although these two specimens are identical in sequence, they differ from a published sequence from a European specimen by 15%, indicating significant undocumented genetic variation in the natal range of *O. villosa*. Additional sampling of Nearctic populations of *O. villosa* might reveal the same situation reported herein for *O. cincta*.

The hexapod class Collembola consists of small, primitively wingless hexapods with an ancient worldwide distribution (Hopkins 1997). Commonly known as springtails, they primarily feed on lichens and fungal hyphae and are an important part of the soil formation process (Hopkins 1997). The springtail genus *Orchesella* has a Holarctic distribution, with members found in North America, Europe, and Western Asia (Stach 1960, Frati et al. 1992). Of the Old World members, two species have been accidentally introduced to North America: *O. villosa* (L.) and *O. cincta* (L.). It is thought that both species were originally introduced to North America in the ballast of early European ships, or when agricultural plants or products were transported to the New World (Maynard 1951); probably both species have been introduced repeatedly since then. Both *O. cincta* and *O. villosa* have since become established and widespread but spotty in North America (Christiansen and Bellinger 1998), most often in disturbed habitats of the northern United States and southern Canada along coasts and near seaports. *O. cincta* has also been reported from Portland, Oregon (Scott 1942), where native Nearctic congeners apparently are absent (Christiansen and Bellinger 1998).

Timmermans et al. (2005) used the mitochondrial gene cytochrome oxidase subunit II (*cox2*) to study the genetic relationships among different populations of *O. cincta* in Europe. Their findings suggested there were three main population groups within the native range of *O. cincta* (northwestern Europe, central

[1]Department of Entomology and Plant Pathology, The University of Tennessee, 370 Plant Biotechnology Building, 2505 EJ Chapman Drive, Knoxville, Tennessee 37996-4560.

*Corresponding author: e-mail: (jmoulton@.utk.edu).

Europe, Italy). The objective of this research was to use the data of Timmermans et al. (2005) to determine whether the presence of *O. cincta* populations in the Nearctic Region is due to single or multiple introductions. To meet this objective we obtained sequences of cytochrome oxidase II corresponding to the same fragment used by Timmermans et al. (2005) from several Nearctic populations of *O. cincta,* added these sequences to their data, and conducted phylogenetic analyses. A similar analysis was conducted on two specimens of *O. villosa*, one each from Maine and Oregon.

Materials and Methods

Taxon Sampling. Specimens of *Orchesella cincta* were collected from Wisconsin, Minnesota (two sites), Maryland, Michigan, Oregon, and Washington. Specimens of *O. villosa* were collected from Maine and Oregon. Most specimens were aspirated from under the bark of decaying logs, collected by sifting leaf litter, or beaten from lichen-covered branches; the Maryland specimen of *O. cincta*, was extracted from leaf litter samples by means of a Tullgren funnel. Voucher specimens and cuticles were deposited at the University of Tennessee (ECB research collection). When multiple specimens were collected (Minnesota, Wisconsin, Michigan, Oregon), the largest individual was photographed prior to total DNA extraction for vouchering puposes. Outgroups added to root the phylogeny included *Dicranocentrus marias* Wray and *Heteromurus major* (Moniez 1889), several Nearctic *Orchesella* species, viz., *Orchesella hexfasciata* Harvey, *Orchesella gloriosa* Snider, and *Orchesella annulicornis* Mills, and the closely related European species *O. villosa* (represented by the Maine and Oregon specimens). Collection information and GenBank accession numbers for all specimens are given in Table 1.

Laboratory Methods. Total DNA was extracted using the ThermoScientific GeneJET Genomic DNA Extraction (ThermoScientific, Waltham, MA) kit following the manufacturer's suggested protocol, except for the final step where the DNA was eluted in 70µL of elution buffer warmed to 52°C. Purified DNA samples were stored at -20°C. We used the forward primer used by Timmermans et al. (2005): TL2-J-3037 (5'-AATATGGCAGATTAGTGC-3') (Simon et al. 1994) and a custom reverse primer designed by JKM (COII-R: 5'-CCACAGATTTCTGAGCATTGACC-3'), which amplify a 563 base pair fragment from the mitochondrial genome comprising the 3' end of the tRNA gene encoding for the amino acid leucine (i.e., the first 10 bases of polished final sequences) and the ca. 5' half of cytochrome oxidase II (*cox2*). This gene region has been previously and successfully used for phylogenetic purposes in Collembola (Frati and Carapelli 1999; Frati et al. 2001; McGaughran et al. 2010; Luque et al. 2011).

Amplifications were performed in GenePro (Bioer Technology Co., Hangzhou, China) thermal cyclers, using 0.2µL TaKaRa Ex Taq Hotstart DNA polymerase (Takara Bio, Shiga, Japan) and the following components: 36µL ddH_2O, 5µL 10x buffer, 2µL $MgCl_2$, 3.5µL dNTPs, 1.3µL template DNA, and 3µL (7µM) of forward and reverse primer. Thermal cycling parameters were as follows: 1.5 min denaturation soak at 94°C; 5 cycles of 94°C for 30s, 52°C for 30 s, and 72°C for 45s; 15 cycles of 94°C for 30s, 47°C for 25s, and 72°C for 45s; 35 cycles of 94°C for 30s, 42°C for 20s, 72°C for 45s, 72°C soak for 3 min, and 13°C hold. PCR products were electrophoresed in 1% agarose, excised from the gel, and purified using a QiaQuick Gel Extraction Kit (Qiagen, Valencia, CA). The reverse strand of each product was cycle sequenced in 20µL reactions using 16-fold diluted Big Dye 3.1 (Applied Biosystems, Foster City, CA). Sequencing reactions were cleaned using Centrisep columns (Prinecton Separations, Adelphia, NJ) and dried in a Centrivap Concentrator (LABCONCO, Kansas City, MO). Sequencing of samples was performed by the University of Tennessee-Knoxville Molecular Biology Resource Facility. Sequences were verified for accuracy using Sequencher 4.7 (GeneCodes, Ann Arbor, MI).

https://scholar.valpo.edu/tgle/vol48/iss3/1
DOI: 10.22543/0090-0222.1014

Table 1. Sources of North American specimens used in this study.

Taxa	Country	State	County	GB Accession #
Heteromerus major	USA	Oregon	Lane	KP748426
Dicranocentrus maria	Puerto Rico	N/A	Isabela	KP748425
Orchesella gloriosa	USA	Tennessee	Sevier	KP748423
Orchesella annulicornis	USA	Illinois	Will	KP748387
Orchesella hexfasciata	USA	Maine	Hancock	KP748396
Orchesella villosa	USA	Oregon	Lane	KP843123
Orchesella villosa	USA	Maine	Hancock	KP748454
Orchesella cincta	USA	Michigan	Alger	KP748453
Orchesella cincta	USA	Oregon	Lane	KP748451
Orchesella cincta	USA	Washington	Clallam	KP843122
Orchesella cincta	USA	Minnesota	Hennepin[a]	KP748449
Orchesella cincta	USA	Wisconsin	Taylor	KP748450
Orchesella cincta	USA	Maryland	Anne Arundel	KP748453
Orchesella cincta	USA	Minnesota	Clearwater[b]	KP843120

[a] Minneapolis-St. Paul environs
[b] Lake Itasca State Park

DNA Alignment and Phylogenetic Analysis. Sequences from all European populations of *O. cincta* reported in Timmerman et. al (2005) were added to our data matrix of Nearctic *O. cincta* populations plus selected outgroups. Alignment of the sequences was straightforward, requiring no indels. Mesquite (Maddison and Maddison 2011) was used to partition the data set into codon positions. PAUP* (Swofford 2001) was used to calculate pairwise sequence divergence. HKY85 corrected distances were calculated because this model was selected as the best fit by jModelTest (see below).

Bayesian and maximum likelihood analyses were performed on the nucleotides. The optimal evolutionary model was determined using jModelTest 2.1.4 (Guindon and Gascuel 2003; Darriba et al. 2012), which selected the HKY+I+G as the best fit model based on the Bayesian information criterion [-lnL=3885.2396;K=54; BIC=8112.4764; f(A)=0.3358; f(C)=0.1979; f(G)=0.1087; f(T)=0.3577; Ti/tv=4.7769;I=0.4620; G=1.018].

Best-fit models were implemented in Bayesian analyses using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003). Every Markov chain in the Bayesian search was started from a random tree and set to compute 1×10^7 generations, sampling every 1000^{th} one from the chain, resulting in a total of 1000 trees. Three hot chains and 1 cold chain were run simultaneously, with pre-stationarity trees discarded as burn-in. Each simulation was run twice. Default settings for the priors were used, and the base frequencies were estimated from the data. Tracer 1.6 (Rambaut et al. 2013) was used to parse and combine the log files, determine at which point the Markov Chain Monte Carlo (MCMC) began to sample from the stationarity distribution, and to check that effective sample sizes (ESSs) were sufficient for all parameters. To reduce the probability of convergence on local optima, multiple starting points for each chain were used. Maximum likelihood analysis was performed using RAxML-HPC2 (Stamatakais 2006; Stamatakis et al. 2008), as implemented in CIPRES-XSEDE (Miller et al. 2010). Analyses were conducted using the evolutionary model GTRGAMMAI (HKY+I+G) as well as the default model (GTRCAT), each with 1,000 bootstrap replicates, with the data partitioned by codon position and gene. There were no discernible differences between approaches. Bayesian posterior probabilities and nonparametric bootstrap proportions were used to assess node support (Felsenstein 1985).

Results

mtDNA Data Set Properties. The analyzed data matrix was comprised of 25 taxa, including 2 distal outgroups, 4 proximal outgroups from the Nearctic region, 2 proximal outgroups from Europe (1 specimen of *O. villosa* from Maine that was identical in sequence to a specimen taken in Oregon and 1 from Europe (Carapelli et al. 2007), 7 Nearctic individuals of *O. cincta*, and 11 specimens of *O. cincta* from Europe (Timmermanns et al. 2005). *Cox2* sequences for the newly acquired North American samples are deposited in GenBank (Table 1).

Percent pairwise divergence between distal outgroups ranged from 30.4 (*H. major* and *D. marias*) to 38.9 (*H. major* and *O. gloriosa*). Percent pairwise divergence between *O. villosa* and *O. cincta* populations ranged from 21.3 (*O. villosa* Maine and *O. cincta* Oregon) to 28.7 (*O. villosa* Europe and Italy 3). Percent pairwise divergence within populations of *O. cincta* ranged from 0.0% (12 comparisons) to 19.5 % (Italy 1 and Italy 3). Pairwise divergence between USA and European specimens of *O. villosa* was 15.2%.

The North American specimens of *O. cincta* from Oregon, Washington, and Minnesota were identical to published sequences from specimens examined by Timmermans et al. (2005) from northwestern Europe (NW Europe 1 and NW Europe 6). Our Michigan *O. cincta* specimen differed from the Timmermans et al. (2005) Italy 5 specimen by 1.1% divergence.

https://scholar.valpo.edu/tgle/vol48/iss3/1
DOI: 10.22543/0090-0222.1014

Results of Phylogenetic Analyses. With *H. major* selected as the root *D. marias* was the sister group to *Orchesella* spp. The three Nearctic representatives of *Orchesella* were strongly supported (BS = 97, PP = 1.0) as a monophyletic group and formed the sister group to a weakly supported *O. villosa* plus *O. cincta* clade. Monophyly of *O. cincta* was strongly supported (BS = 100, PP = 1.0). Within *O. cincta*, Italy 1 formed the sister group to all remaining sampled populations, although with weak support. A strongly supported (BS = 91, PP = 1.0) clade of three Italian *O. cincta* populations (Italy 3, Italy 4, and Italy 6) formed the sister group to Italy 5 plus Michigan, which were strongly supported as sister taxa (BS = 100, PP = 1.0) and in turn were sister to a well-supported (BS=94, PP=0.99) clade comprised of all remaining sampled *O. cincta* specimens (Fig. 1).

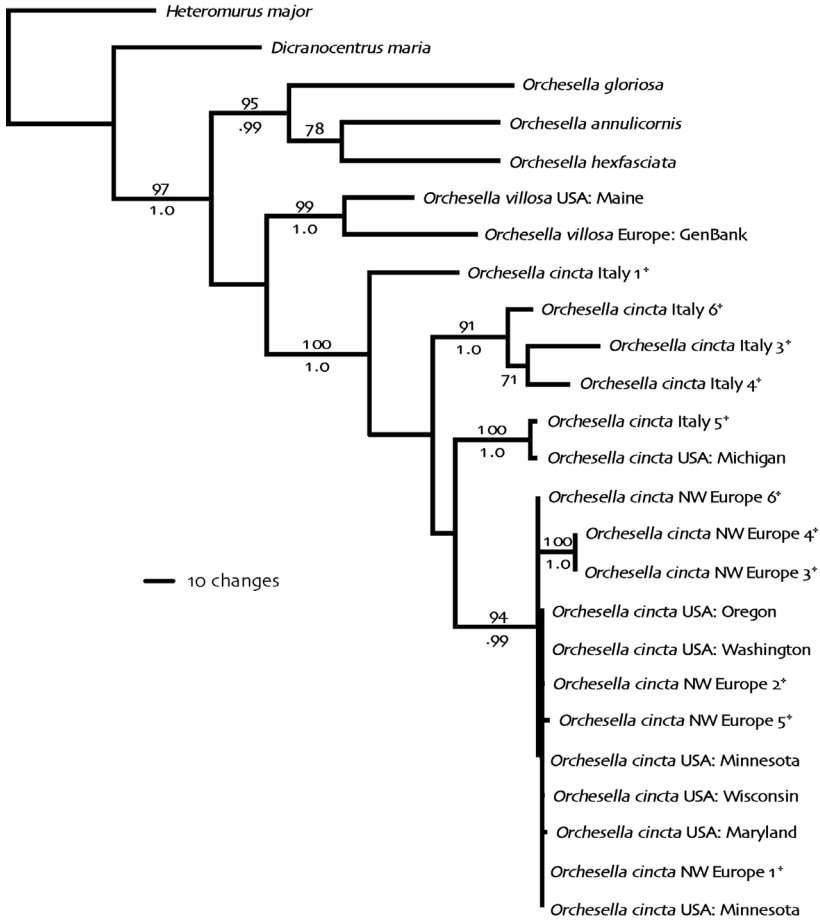

Figure 1. Phylogram depicting relationships inferred from maximum likelihood analysis (RaxML, GTR+I+G) of nucleotides from portions of mitochondrial genes encoding tRNA-leucine and cytochrome oxidase II. Maximum likelihood nonparametric bootstrap scores and Bayesian posterior probabilities shown above and below nodes, respectively. An asterix denote samples examined by Timmermans et al. (2005).

Discussion

With one exception, all sampled North American *O. cincta* specimens matched up closely with an assemblage of weakly or undifferentiated populations found in northwestern Europe. Immigration patterns of cultural groups from this region of Europe into the U.S. (Anderson and Blanck 2012) would predict such an occurrence. Migration of humans and intercontinental trade of agricultural and horticultural goods from the Upper Midwest to the West Coast are the most likely explanation for western movement of newly established northwestern European *O. cincta* populations in the Nearctic. The one exception among the Midwestern USA populations was the specimen collected from Michigan, which paired strongly with a specimen Timmermans et al. (2005) examined from Italy (Italy 5). International shipping through the Saint Lawrence River/Great Lakes system to the large ports in Michigan could explain how this population was introduced. The presence of two distinct populations of *O. cincta* in the United States clearly indicates this species may have been introduced multiple times from different parts of its native range.

The considerable genetic distance (i.e, 75 base pairs, 15.2% pairwise divergence) between the *O. villosa* specimens collected in Maine and Oregon, which were identical in sequence, and the specimen from which the published *O. villosa* mitochondrial genome (GB# NC_010534) was generated, is on par with observations made by Frati et al. (2000), who noted large amounts of differentiation among native populations of this species. A thorough phylogeographic study of this species in its native established ranges might yield data similar to what we present here for *O. cincta*.

The results reported here do not agree fully with the conclusions of Porco et al. (2013), whose analysis of barcodes for Canadian *O. cincta* and *O. villosa* versus European specimens suggested similar genetic structure of European and North American populations due to massive and multiple introductions. Instead, it appears that both species have been introduced repeatedly but different haplotypes occur only at widely scattered sites.

Orchesella cincta is common throughout Europe (Stach 1960) and sometimes more abundant in forest than any other springtail species (van Straalen 1989). The potential effects of *O. cincta* in North America on native springtail populations are unknown but not likely to be of consequence, as the species has been present on the continent for at least 140 years without becoming widespread. Other much more recently introduced species (e.g., *Homidia socia* Denis and *Lepidocyrtus paradoxus* Uzel) now occur over much of the eastern half of temperate North America (see records in Christiansen & Bellinger 1998). Packard (1873) noted *O. cincta* (as *O. flavopicta* Packard) from Massachusetts. Since then it has been verified from U.S. localities stated in this paper as well as from coastal Maine and Vermont (Christiansen & Bellinger 1998), and from the Canadian provinces of New Brunswick, Newfoundland, Nova Scotia, and Ontario (Porco et al. 2013). Its apparent preference for cooler regions, its large size, and ease of identification make it a potentially good indicator species for assessment of climatic warming.

Acknowledgments

We thank Felipe Soto-Adames for providing specimens of *Dicranocentrus marias*. This work was supported by the Tennessee Agricultural Experiment Station and a professional development grant from the University of Tennessee Graduate School.

Literature Cited

Anderson P. J. and D. Blanck. 2012. Norwegians and Swedes in the United States. Minnesota Historical Society Press. St. Paul, Minnesota.

Carapelli A, P. Lió, F. Nardi, E van der Wath, and F. Frati. 2007. Phylogenetic analysis of mitochondrial protein coding genes confirms the reciprocal paraphyly of Hexapoda and Crustacea. BMC Evolutionary Biology, 7 (Suppl 2): S8.

Christiansen K. and P. Bellinger. (1998) The Collembola of North America, north of the Rio Grande: A taxonomic analysis. Grinnell College. Grinnell, Iowa.

Darriba, D, G. L. Taboada, R. Doallo, and D. Posada. 2012. jModeltTest2: more models, new heuristics and parallel computing. Nature Methods 9: 772.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

Frati, F. and A. Carapelli. 1999. An assessment of the value of nuclear and mitochondrial genes in elucidating the origin and evolution of *Isotoma klovstadi* Carpenter (Insecta, Collembola). Antarctic Science 11: 160-174.

Frati, F, P. P. Faniciulli, and R Dallai. 1992. Genetic diversity and taxonomy in soil-dwelling insects: the genus *Orchesella*. Journal of Heredity 83: 275-281.

Frati, F., E. Dell'Ampio, S. Casasanta, A. Carapelli, and P. P. Fanciulli. 2000. Large amounts of genetic divergence among Italian species of the genus *Orchesella* (Insecta, Collembola) and the relationships of two new species. Molecular Phylogenetics and Evolution 17: 456-461.

Frati, F., G. Spinsanti and R. Dallai. 2001. Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence for population differentiation. Polar Biology 24: 934-940.

Guindon, S and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. Systematic Biology 52: 696-704.

Hopkin, S. P. 1997. Biology of the springtails (Insecta: Collembola). Oxford University Press.

Luque, C, L. Legal, P. Winterton, N. A Mariano, and C. Gers. 2011. Illustration of the structure of arthropod assemblages (Collembola and Lepidoptera) in different forest types:An example in the French Pyrenees. Diversity 3: 693-711.

Maddison, W. P.and D. R. Maddison. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.72. Available from: http://mesquiteproject.org (accessed December 2015).

Maynard, E. A. 1951. A Monograph of the Collembola or springtail insects of New York State. Comstock Publishing Co. Ithaca, New York.

McGaughran, A, G. Torricelli1, A. Carapelli, F. Frati, M. I. Stevens, P. Convey, and I. D. Hogg. 2010. Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. Journal of Biogeography 37: 107-119.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 NOV 2010, New Orleans, LA.

Packard, A. S. 1873. Synopsis of the Thysanura of Essex County, Mass., with descriptions of a few extralimital forms. Annual Report of the Trustees of the Peabody Academy of Science 1873: 23-51.

Porco, D., T. Decaëns, L. Deharveng, S. W. James, D. Skarzyinski, C. Erséus, K. R. Butt, B. Richard, and P. D. N. Hebert. 2013. Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America. Biological Invasions 15: 899-910 (Supplemental data, Table S1).

Rambaut, A., M. Suchard and A. J. Drummond. 2013. Tracer v1.6, Available from: http://tree.bio.ed.ac.uk/software/tracer/ (accessed December 2015).

Ronquist, F.and J. P. Huelsenbeck. 2003. MrBayes 3:Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Scott, D. B. 1942. Some Collembola records for the Pacific Coast and a description of a new species. Panpacific Entomology 18:177-186.

Simon, C., F. Frati, A. T. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651-701.

Stach, J. 1960. The apterygotan fauna of Poland in relation to the world fauna of this group of insects. Tribe: Orchesellini. Pastwowe Wydawnictwo Naukowe, Kraków.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688-2690.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML Web Servers. Systematic Biology 57: 758-771.

Swofford, D. L. 2001. PAUP*: Phylogenetic analysis using Parsimony (and Other Methods). Version 4.0b10. Sinauer, Sunderland, MA.

Timmermans, M. J. T. N., J. Ellers, J. Mariën, S. C. Verhoef, E. B. Ferwerda, and N. M. van Straalen. 2005. Genetic structure in *Orchesella cincta* (Collembola): strong subdivision of European populations inferred from mtDNA and AFLP markers. Molecular Ecology 14: 2017-2024.

Van Straalen, N. M. 1989. Production and biomass turnover in two populations of forest floor Collembola. Netherlands Journal of Zoology, 39: 156–168.