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DNA sequences identify invasive alien *Cardamine* at Lake Constance

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ABSTRACT

The shores of Lake Constance in southwest Germany inhabit an endemic and highly endangered lake-shore community. In spring 2004, a so far unknown *Cardamine* (Brassicaceae) was detected at the lake-shores. In the subsequent years, this taxon has spread rapidly, 95 locations have been recorded until spring 2007. We tested the utility of DNA sequences to distinguish between two alternative hypotheses regarding the appearance of this new invasive taxon: a local formation via hybridization between native species versus an introduction of a non-native taxon. The relative DNA contents was analysed as an additional independent character. DNA sequences provided substantial evidence that the unknown *Cardamine* taxon, recently discovered at Lake Constance in southwest Germany, is a non-native species introduced from abroad. Sequences of the internal transcribed spacer of the large subunits of nuclear ribosomal DNA and two noncoding regions of chloroplast DNA (*trnL* intron, *trnL/F* spacer) were distant from sequences of all native *Cardamine* species providing evidence against a hybridization hypothesis. In contrast, DNA sequences of the unknown *Cardamine* were identical to one accession of *Cardamine flexuosa* auct. non With. (Asian *C. flexuosa*) from Japan. The introduction history of Asian *C. flexuosa* at Lake Constance and the potential threat to the native lake-shore vegetation is discussed. Our study highlights the potential of DNA sequences to identify invasive genotypes and source regions.

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1. Introduction

Lake Constance (southwest Germany) is the largest northern pre-alpine lake with a surface area of 536 km² and a shore length of 273 km. It is distinguished from most other pre-alpine lakes by its natural water-level fluctuation with highest water levels during late spring and early summer (Jöhnk et al., 2004). The exposed parts of the lake-shore contain an amphibious plant community phytosociologically described as *Deschampsietum rhenanae* (Lang, 1990). This community is characterized by narrow endemic species, mainly *Myosotis*

rehsteineri Wartm., *Deschampsia littoralis* (Gaudin) Reut., and other rare amphibious plants (*Littorella uniflora* (L.) Asch., *Ranunculus reptans* L.). The small herbaceous and perennial species are weak competitors but are well adapted to the seasonal water-level fluctuations of Lake Constance (Peintinger et al., 2007). The occurrence of the *Deschampsietum* has been reduced dramatically during the last 100 years (Dienst and Strang, 2002; Dienst et al., 2004; Strang and Dienst, 2004). In spring 2004, a so far unknown *Cardamine* (Brassicaceae) was detected at the shores of Lake Constance. In the subsequent years, this taxon (called *C. "Lake Constance"* throughout the

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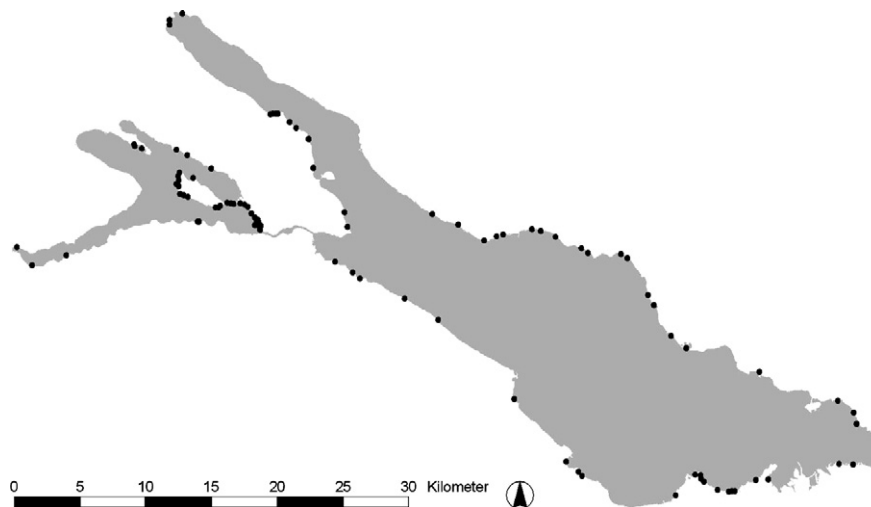


Fig. 1 – Distribution of Asian *Cardamine flexuosa* at Lake Constance (southwest Germany) in spring 2007.

paper) has spread rapidly and invaded the native lake-shore communities, 95 locations have been recorded until spring 2007 (Fig. 1). The unknown *Cardamine* resembled the native species *Cardamine hirsuta* L. and *Cardamine flexuosa* With., both successful colonizers, but had no basal leaf rosette. Photographs and plants from Lake Constance have been examined by a number of specialists but without a clear result. Two hypotheses were formulated regarding the origin of the unknown plants: (1) The plants could be of recent hybrid origin. *Cardamine* species occurring at the shores of Lake Constance are *C. hirsuta* ($2n = 2x = 16$), *C. flexuosa* ($2n = 4x = 32$), and *Cardamine pratensis* L., which occurs in different ploidy levels ($2n = 2x - 6x$). Hybrids were reported between *C. hirsuta* and *C. flexuosa* (*C. ×zahlbruckneriana* O. E. Schulz) and between *C. flexuosa* and *C. pratensis* (*C. ×haussknechtiana* O. E. Schulz). However, none of these hybrids has been reported to reproduce successfully in Central Europe. (2) As a second hypothesis, the plants could have been introduced from abroad. Putative source regions would be North America or Eastern Asia, which both have a high number of native and also endemic *Cardamine* species. *Cardamine* in eastern Asia has only recently been subject to detailed molecular systematic studies (Lihova et al., 2006a).

In order to estimate a potential threat to native biodiversity it is necessary to identify the origin of invasive plant species as early as possible. The ecology of introduced species in their native range may provide important information in an attempt to predict where potential invaders will be successful. DNA sequences provide powerful tools for analysing interspecific hybridization (Franzke and Mummenhoff, 1999; Lihova et al., 2006b) and have recently been suggested as tools for invasive species identification (DNA barcoding, Armstrong and Ball, 2005). It has been shown that DNA sequences commonly used for phylogenetic studies might perform well even though they were not produced to be used as barcodes (Chase et al., 2005). The internal transcribed spacer of the large subunits of nuclear ribosomal DNA and two noncoding regions of chloroplast DNA (*trnL* intron, *trnL/F* spacer) have been used widely for phylogenetic reconstruction in the large genus *Cardamine* (Franzke et al., 1998; Bleeker et al., 2002a; Lihova

et al., 2006a). DNA sequences are available for about 150 of approximately 200 *Cardamine* species worldwide. For many species multiple accessions are available. Ploidy analyses are also a useful tool to distinguish between different taxonomic entities in genera which are rich of polyploids like *Cardamine* Lihova et al. (2007) or the closely related genus *Rorippa* (Bleeker and Matthies, 2005).

In this paper we test the utility of DNA sequences to distinguish between two alternative hypotheses regarding the appearance of a new invasive taxon: a local formation via hybridization between native species versus an introduction of a non-native taxon. The relative DNA contents was analysed as an additional independent character.

2. Materials and methods

2.1. Origin of plant material

In spring 2006, we visited six locations of *C. “Lake Constance”* at western parts of the lake. At five of these locations, *C. “Lake Constance”* co-occurred with other *Cardamine* species. Seven morphological characters (height of stem, length of petals, length of sepals, length of middle stem leaf, length of first lateral leaflet of middle stem leaf, presence/absence of stem hairs, presence/absence of basal leaf rosette) were used to evaluate morphological variation of *Cardamine* taxa at the lake shore. *C. pratensis* could easily be distinguished by its large petals. *C. “Lake Constance”* was characterized by lacking a basal leaf rosette, all other quantitative morphological characters analysed were in the range of *C. hirsuta* and *C. flexuosa*. The hairiness of the stem, a character used to distinguish between *C. hirsuta* and *C. flexuosa*, varied in *C. “Lake Constance”*, but in most plants the stem was less hairy than in *C. flexuosa* (Klausmeyer, 2006). The number of stamens in *C. “Lake Constance”* was six like in *C. flexuosa*.

2.2. Ploidy analysis

Flow cytometry was used for the determination of relative DNA amount. Fresh leaf material was harvested in the field

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