First report of dodder (Cuscuta japonica) parasitizing Japanese red pine (Pinus densiflora) in China

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Abstract
Dodders (Cuscuta spp.) are widespread parasitic weeds on many plants all over the world, which can lead to considerable damage to ecosystems (Costea et al. 2011). Many Cuscuta species are recognized as Class 1 designated Prohibited Noxious Weeds (Weed Seeds Order, 2016), as they can reduce the biomass of hosts and modify the structure of plant communities (Pennings and Callaway, 2002). Cuscuta japonica is a hemiparasitic plant with many hosts including Araliaceae, Asteraceae, Fabaceae, Polygonaceae, Rutaceae, and Salicaceae (Chen et al. 2012; Yuan et al. 2015). Until now, C. japonica has not been reported parasitizing hosts from gymnosperm species. In September 2019, the dodder was observed parasitizing Pinus densiflora in Lushan Mountain (36°18′17.99″N, 118°5′50.27″E, elevation 662m), Zibo City, Shandong Province, China. P. densiflora, commonly known as Japanese red pine, is an evergreen pine with a range including Japan, the Korean Peninsula, and northeastern China. It is widely cultivated due to its timber for construction, trunk for resin, bark for tannin, and needles for medicine. One individual of P. densiflora, approximately 5 m tall, was infected, and the branches and needles of the host were wrapped by the leafless stems of this dodder. The haustoria penetrating branches and needles of the host were visible to the naked eye. Uneven holes were emerged on Pinus needle after removal of the dodder stem, and the tissues of Pinus needle around the holes become black. Some needles showed chlorotic, wilted, and even defoliated. This dodder can be morphologically identified as C. japonica according to previous investigation records and descriptions, including slightly yellow stems with branches 1.4-2.6 mm in diameter,
capsules globose and smooth, style 1, and stigma 2-lobed with elongated lobes. An area of ca. 5.8 ha around the infected *P. densiflora* was carefully examined for this dodder, and only three infections were discovered on one individual of *P. densiflora*. No dodders were found on the nearby deciduous trees within above examined area. However, this dodder was found parasitizing deciduous trees like *Ulmus pumila* outside of the examined area in the Lushan mountain, so *P. densiflora* might not be a primary host for this dodder from the point of view of the entire mountain. Therefore, we tentatively classify *P. densiflora* as a rare host of this dodder. Species identification was further conducted by characteration of the whole plastome. First, total DNA was extracted from silica-dried stems of this dodder. Then genome-skimming method was applied to generate the whole plastome as described in Qu et al. 2019. One sequence of 121,005 bp were deposited in GenBank (accession number: MN585289). BLAST analysis reconfirmed the identity of this dodder as *C. japonica* (99.92%, target accession number: MH780080). Furthermore, phylogenetic analysis supported this dodder as sister to *C. japonica* with nearly same branch length (0.0002 vs. 0.0003) with 100% bootstrap support value. Thus, this dodder observed in Zibo City was identified as *C. japonica* based on evidence from morphology, sequence, and phylogeny. Specimens of *C. japonica* on *P. densiflora* were collected and deposited at the herbarium of College of Life Sciences, Shandong Normal University (No. 092217A). *C. japonica* seems to be an extremely rare occurrence on *P. densiflora*. It will need more evidence to judge if it is a real infection on *P. densiflora*. It is probably that *C. japonica* is not a parasite that can threaten populations of *P. densiflora* at present. Therefore, *C. japonica* is not considered as a management concern on Japanese red pine. To the best of our knowledge, this is the first report of *C. japonica* parasitizing *P. densiflora*, and the only known example of *C. japonica* parasitizing a gymnosperm species in the world.

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References

Figure S1. Occurrence and symptoms of Pinus densiflora parasitized by Cuscuta japonica in the field. (A) three infections found on one individual of P. densiflora (white arrow); (B) mild infection (white arrow); (C) functional haustoria formation on the branch of P. densiflora (white arrow); (D) two needles of P. densiflora were simultaneously parasitized by one stem of C. japonica, one needle was normal (red arrow), but the other one was wilt or dead (yellow arrow); (E) one needle parasitized by C. japonica was normal (red arrow), but another one infected by C. japonica was wilt or dead (yellow arrow).

Figure S2. Maximum likelihood phylogenetic tree of Cuscuta species with whole plastomes using all protein-coding genes. The tree was reconstructed by RAxML v8.2.10 in CIPRES Science Gateway (https://www.phylo.org/), including tree robustness assessment using 1,000 rapid bootstrap replicates with the GTRGAMMA substitution model. Ipomoea obscura and I. tricolor were used as outgroup. GenBank accession numbers were shown behind species names. C. japonica in this study was indicated by bold font. The numbers on branches were bootstrap support values.