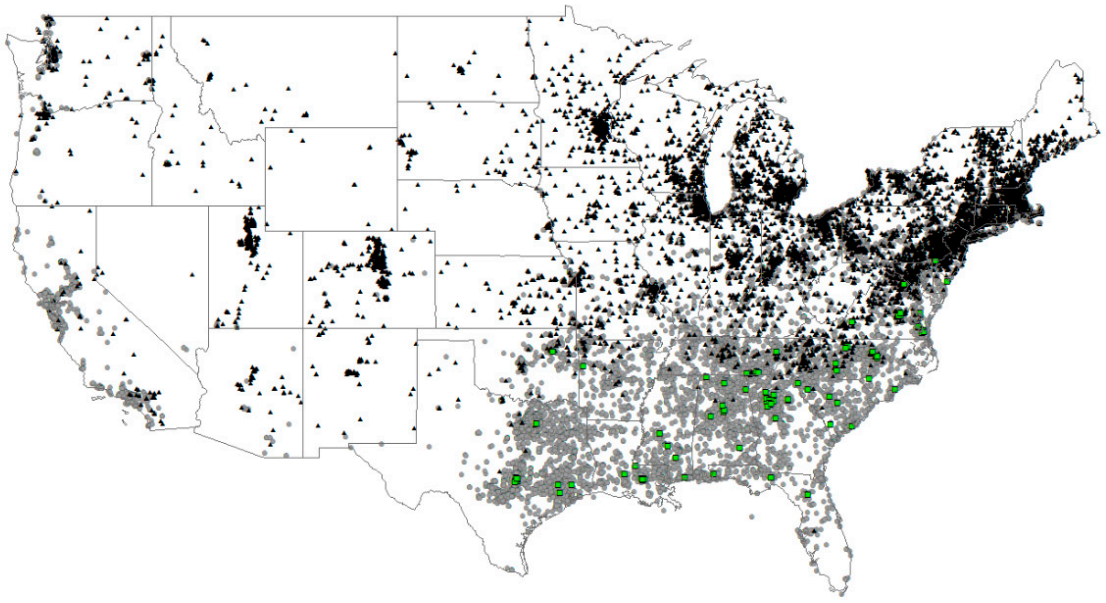

Figure S1. Distribution map of *O. ligustri, Ligustrum* spp., and *Syringa* spp. in the US. Green squares represent *O. ligustri*, gray dots represent *Ligustrum* spp., and black triangles represent *Syringa* spp.

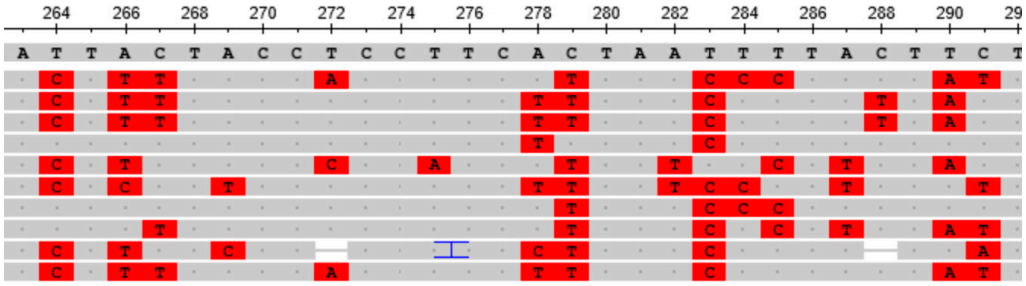

Figure S2. Gene region of a newly developed forward COI primer within 658-bp sequence of *O. ligustri* (263 to 289). MSA viewer showed the gene region is interspecifically highly diverse (red color).

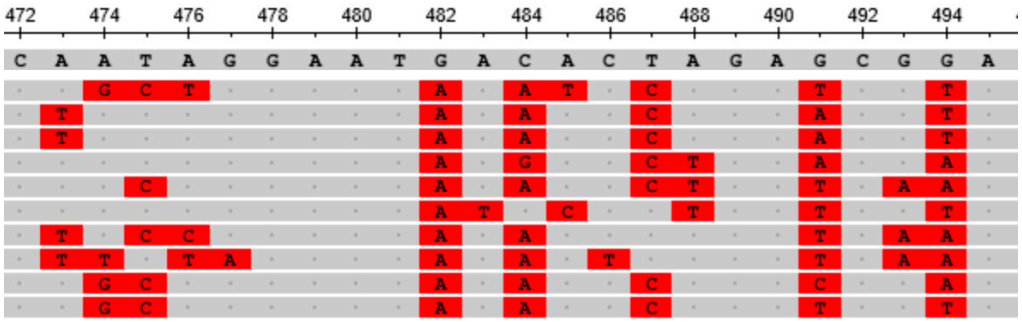

Figure S3. Gene region of a newly developed reverse COI primer within 658-bp of of *O. ligustri* (473 to 494). MSA viewer showed the gene region is interspecifically highly diverse (red color).

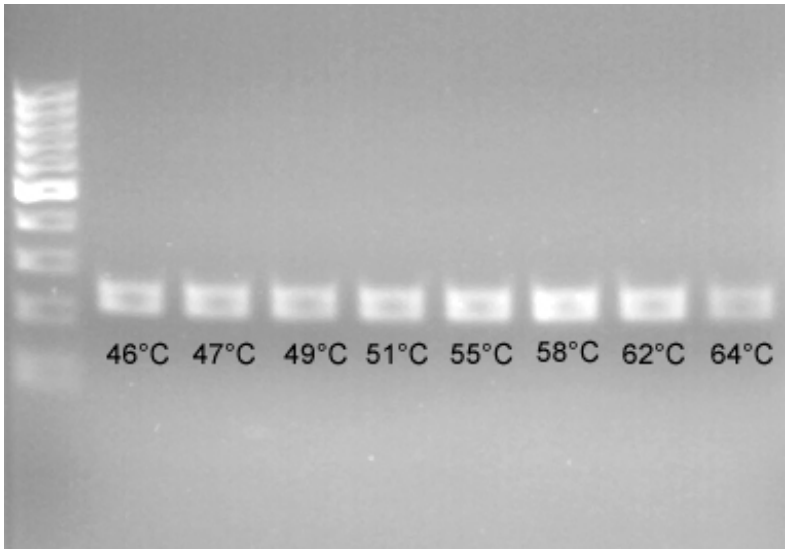

Figure S4. Gradient PCR results indicated that the newly developed species-specific primers worked best at the 58°C annealing temperature.

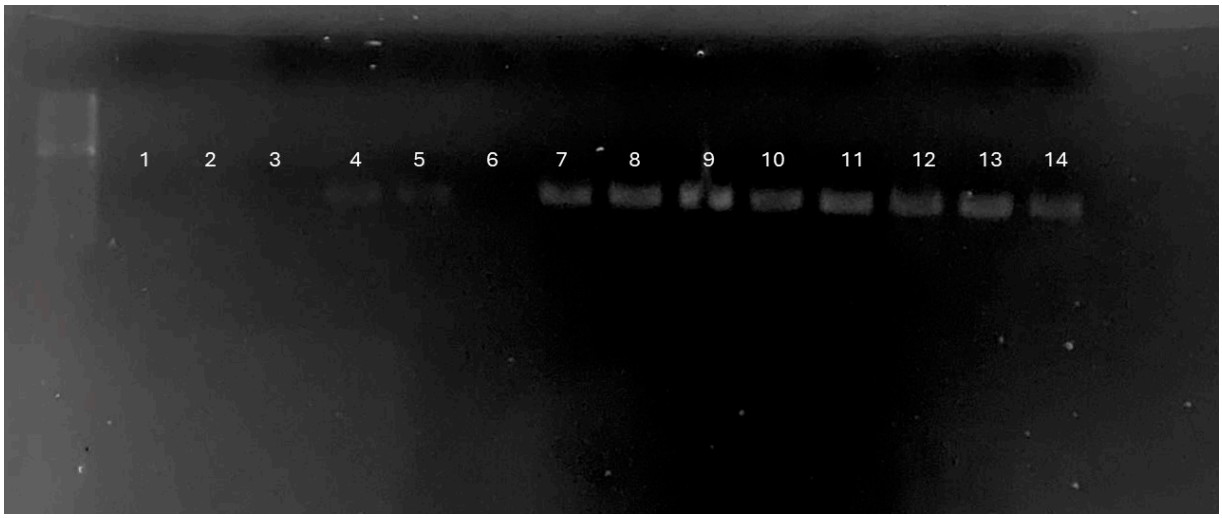

Figure S5. DNA detection results of *O. ligustri* from fruits and adults weevils: 1: *L. sinense* fruits (n =10; Houston Arboretum, Texas; absent); 2: *L. sinense* fruits (n = 10, High Isle, Texas; absent) 3: *L. sinense* fruits (n = 5; Tyrell, Texas; absent); 4: *L. sinense* fruits (n =5; Wesson, Mississippi; detected); 5: *L. sinense* berries (n = 10; Kathleen, Georgia; detected); 6: *L. sinense* fruits (n = 10; Sprewell Bluff, Georgia; absent); 7: *L. sinense* fruits (n = 5; Perry, Georgia; detected); 8: *O. ligustri* adult (n = 1; Wesson, Mississippi; detected); 9: *O. ligustri* adult (n = 1; Wesson, Mississippi; detected); 10: *O. ligustri* adult (n = 1; Auburn, Alabama; detected); 11: : *O. ligustri* adult (n = 1; Auburn, Alabama; detected); 12: *O. ligustri* adult (n = 1; Louisiana, control 1; detected); 13: : *O. ligustri* adult (n = 1; Louisiana, control 2; detected); 14: *O. ligustri* adult (n = 1; Louisiana, control 3; detected).

Figure S6. Positions of the newly developed primers on COI DNA barcoding region.

Table S1. Experimental setup to determine presence of *O. ligustri* DNA in *L. sinense* fruits in Louisiana.

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	# 11	# 12	# 13	# 14	# 15	# 16
Number of fruits	1	2	1	1	10	10	10	10	10	10	10	10	10	10	10	10
ATL buffer (µL)	180	180	270	270	900	900	900	900	900	900	900	900	900	900	900	900
Proteinase K solution (µL)	20	20	30	30	100	100	100	100	100	100	100	100	100	100	100	100
AL Buffer (µL)	200	200	200	200	100	100	100	100	100	100	100	100	100	100	100	100
100% EtOH (µL)	200	200	200	200	100	100	100	100	100	100	100	100	100	100	100	100
Fruit group	i	i	i	i	ii	ii	ii	ii	i	i	i	i	iii	iii	iii	iii
Plant species	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch	Ch
Site	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Table S2. Experimental setup to determine presence of *O. ligustri* DNA in three *Ligustrum* spp., *L. sinense* (Ch), *L. lucidum* (Tr) and *L. japonicum* (Ja) fruits in Louisiana.

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	# 11	# 12	# 13	# 14	# 15
Number of fruits	1	2	10	1	2	10	1	2	4	1	2	5	1	2	10
ATL buffer (µL)	180	180	900	180	180	900	180	180	900	180	180	900	180	180	900
Proteinase K solution (µL)	20	20	100	20	20	100	20	20	100	20	20	100	20	20	100
AL Buffer (µL)	200	200	100	200	200	100	200	200	100	200	200	100	200	200	100
100% EtOH (µL)	200	200	100	200	200	100	200	200	100	200	200	100	200	200	100
Fruit group	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii	i & ii
Plant species	Ch	Ch	Ch	Ch	Ch	Ch	Tr	Tr	Tr	Tr	Tr	Tr	Ja	Ja	Ja
Site	A	A	A	C	C	C	C	C	C	B	B	B	B	B	B

Table S3. Experimental setup to determine presence of *O. ligustri* DNA in fruits of non-ligustrum plants, *Ardisia crenata* (Ca), *Sambucus* sp. (El), *Ilex vomitoria* (IL), *Nandina* sp. (Na), *L. sinense* (Ch).

	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	# 11	# 12	# 13
Number of fruits	10	10	10	10	10	10	4	4	10	1	2	10	10
ATL buffer (µL)	900	900	900	900	900	900	900	900	900	180	180	900	900

Proteinase K solution (µL)	100	100	100	100	100	100	100	100	100	20	20	100	100
AL Buffer (µL)	100	100	100	100	100	100	100	100	100	200	200	100	100
100% EtOH (µL)	100	100	100	100	100	100	100	100	100	200	200	100	100
Fruit group	iii	iii	i & ii	i & ii	Dark purple	Dark purple	ii & iii	ii & iii	ii & iii	i	i	i	ii
Plant species	Ca	Ca	Ca	Ca	El	El	IL	IL	Na	Ch	Ch	Ch	Ch
Site	A	A	A	A	A	A	A	A	A	A	A	A	A