**Table S2** Species identified with NCBI Primer-BLAST that could potentially cross-amplify with our C. cognatus, S. alpinus/malma and, T. arcticus primer sets. Default Primer-BLAST settings were used: primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps. at the 3' end, Ignore targets that have 6 or more mismatches to the primer.

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| **C. cognatus primer set** |  |  |
| **Species** | **Location** | **# mismatches** |
| Comephorus baicalensis | Lake Baikal Russia | 2 |
| Cottus rhotheus | Oregon, USA to British Columbia, Canada | 3 |
| Cottus bairdii | Lower 48 USA and E. Canada | 3 |
| Cottus hubbsi | Columbia Basin USA and Canada | 3 |
| Cottus extensus | Bear lake Utah, USA | 3 |
| Cottus bendirei | Origon and Idaho, USA | 3 |
| Cottus hypselurus | Missouri USA | 3 |
| Cottus caeruleomentum | Eastern USA | 3 |
| Comephorus dybowskii | Lake Baikal Russia | 3 |
| Cottus gulosus | Western USA lower 48 | 4 |
| Abalistes stellaris | Tropical Pacific ocean | 4 |
| Cottus baileyi | Eastern USA | 4 |
| Procottus major | Lake Baikal Russia | 4 |
| Procottus jettelesii | Lake Baikal Russia | 4 |
| Cottinella boulengeri | Lake Baikal Russia | 4 |
| Asprocottus pulcher | Lake Baikal Russia | 4 |
| Asprocottus platycephalus | Lake Baikal Russia | 4 |
| Asprocottus herzensteini | Lake Baikal Russia | 4 |
| Asprocottus korjakovi | Lake Baikal Russia | 4 |
| Boops boops | Eastern Atlantic ocean | 4 |
| Myoxocephalus brandtii | Norhtwest Pacific ocean | 4 |
| Myoxocephalus jaok | Norhtern Pacific ocean | 4 |
| Lycodes raridens | Berrin Sea | 4 |
| Myoxocephalus scorpius | Northern Atlantic ocean | 4 |
| Cottus paulus | Alabama, USA | 4 |
| Myoxocephalus polyacanthocephalus | Norhtern Pacific ocean | 4 |
| Paracottus knerii | Russia | 4 |
| Batrachocottus multiradiatus | Lake Baikal Russia | 4 |
| Limnocottus pallidus | Lake Baikal Russia | 4 |
| Limnocottus griseus | Lake Baikal Russia | 4 |
| Limnocottus bergianus | Lake Baikal Russia | 4 |
| Limnocottus godlewskii | Lake Baikal Russia | 4 |
| Cottus ricei | USA lower 48 and E. Canada | 4 |
| Cottus ferrugineus | Europe | 4 |
| Cottus sibiricus | Siberia | 4 |
| Cottus gobio | Europe | 4 |
| Lutjanus fulviflamma | Indian ocean | 5 |
| Cottus carolinae | Southeast USA | 5 |
| Pterogymnus laniarius | Southest Atlantic ocean | 5 |
| Lycodes tanakae | Sea of Japan | 5 |
| Banjos banjos | Western Pacific Ocean | 5 |
| Cottus confusus | Western United States and SW Canada | 5 |
| Pachycara crossacanthum | Southeastern Atlantic ocean | 5 |
| Batrachocottus nikolskii | Lake Baikal Russia | 5 |
| Cyphocottus eurystomus | Lake Baikal Russia | 5 |
| Canthigaster amboinensis | Tropical Pacific ocean | 5 |
| Kuhlia sandvicensis | Tropical Pacific ocean | 6 |
| **S. alpinus/malma primer set** |  |  |
| **species** | **Location** | **# mismatches** |
| Salvelinus taranetzi | Russia | 0 |
| Salvelinus kuznetzovi | Russia | 0 |
| Salvelinus neiva | Russia | 0 |
| Salvelinus krogiusae | Russia | 0 |
| Salvelinus elgyticus | Russia | 0 |
| Salvelinus boganidae | Russia | 0 |
| Salvelinus andriashevi | Russia | 0 |
| Salvelinus albus | Russia | 0 |
| Salvelinus confluentus | Western USA (lower 48) and Canada | 0 |
| Salvelinus schmidti | Russia | 0 |
| Salvelinus kronocius | Russia | 0 |
| Salvelinus leucomaenis | Russia, Korea, Japan | 2 |
| Salvelinus curilus | Korea, Japan | 2 |
| Salvethymus svetovidovi | Russia | 2 |
| Salvelinus fontinalis | Eastern US and Canada. Introduced across N America including few locations in SE Alaska | 3 |
| Salmo trutta | Europe. Introduced throughout the Americas but not Alaska | 3 |
| Salvelinus levanidovi | Western Pacific ocean | 4 |
| Salvelinus namaycush | North America | 4 |
| Barilius bendelisis | South Asia | 5 |
| Salmo platycephalus | Turkey | 5 |
| Salmo obtusirostris | Eastern Europe | 5 |
| Salmo marmoratus | Europe | 5 |
| Odontesthes bonariensis | Argentina | 5 |
| Salmo salar | Atlantic Ocean | 5 |
| Salmo akairos | Morocco | 6 |
| Salmo ohridanus | Eastern Europe | 6 |
| **T. arcticus primer set** |  |  |
| **Species** | **Location** | **# mismatches** |
| Thymallus mertensii | Russia | 0 |
| Thymallus thymallus | Europe and Russia | 0 |
| Thymallus baicalolenensis | Russia | 1 |
| Thymallus burejensis | Russia | 1 |
| Thymallus tugarinae | Russia | 1 |
| Thymallus brevirostris | Russia and Mongolia | 1 |
| Thymallus grubii | Russia | 1 |
| Thymallus yaluensis | Korea | 1 |
| Pomatoschistus marmoratus | Eastern Atlantic ocean | 3 |
| Mystus nigriceps | SE Asia | 3 |
| Prototroctes maraena | Australia | 3 |
| Liopropoma olneyi | Caribbean Sea | 3 |
| Spea bombifrons | Western US and Mexico | 4 |
| Lagocephalus laevigatus | Atlantic ocean | 4 |
| Lethrinus haematopterus | Atlantic ocean | 4 |
| Lagocephalus gloveri | Indo pacific ocean | 4 |
| Lagocephalus cheesemanii | Indo pacific ocean | 4 |
| Lagocephalus spadiceus | Indo pacific ocean | 4 |
| Menticirrhus nasus | Eastern Pacific ocean | 4 |
| Etheostoma simoterum | SE USA | 4 |
| Lagocephalus guentheri | Persian gulf | 4 |
| Pomatoschistus norvegicus | Eastern Atlantic ocean | 5 |
| Ammodytes heian | North Pacific ocean | 5 |
| Amphiprion ocellaris | Western Pacific ocean | 5 |
| Western Pacific ocean | Western Pacific ocean | 5 |
| Pristipomoides typus | Western Pacific ocean | 5 |
| Pseudocoris bleekeri | Western Pacific ocean | 5 |
| Pomatoschistus minutus | Eastern Atlantic ocean | 5 |
| Synagrops spinosus | Western Atlantic ocean | 5 |
| Pomatoschistus microps | Eastern Atlantic ocean | 5 |
| Grammistops ocellatus | Indo pacific ocean | 5 |
| Lagocephalus | Atlantic , Indian, and Pacific oceans | 5 |
| Gobius fallax | Eastern Atlantic ocean | 6 |
| Sebastiscus marmoratus | Western Pacific ocean | 6 |
| Sebastiscus tertius | Western Pacific ocean | 6 |
| Pomatoschistus lozanoi | Eastern Atlantic ocean | 6 |
| Calamus arctifrons | Western Atlantic ocean | 6 |
| Aphonopelma eutylenum | Western United States | 6 |