1 A random forest approach for predicting the presence of *Echinococcus multilocularis*

2 intermediate host Ochotona spp. presence in relation to landscape characteristics in western

3 China

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16 Abstract

17 Understanding distribution patterns of hosts implicated in the transmission of zoonotic disease remains a key goal of parasitology. Here, random forests are employed to model spatial 18 19 patterns of the presence of the plateau pika (Ochotona spp.) small mammal intermediate host 20 for the parasitic tapeworm *Echinococcus multilocularis* which is responsible for a significant 21 burden of human zoonoses in western China. Landsat ETM+ satellite imagery and digital 22 elevation model data were utilized to generate quantified measures of environmental 23 characteristics across a study area in Sichuan Province, China. Land cover maps were 24 generated identifying the distribution of specific land cover types, with landscape metrics 25 employed to describe the spatial organisation of land cover patches. Random forests were used 26 to model spatial patterns of Ochotona spp. presence, enabling the relative importance of the 27 environmental characteristics in relation to Ochotona spp. presence to be ranked. An index of 28 habitat aggregation was identified as the most important variable in influencing Ochotona spp. 29 presence, with area of degraded grassland the most important land cover class variable. 71% of 30 the variance in Ochotona spp. presence was explained, with a 90.98% accuracy rate as 31 determined by 'out-of-bag' error assessment. Identification of the environmental characteristics 32 influencing Ochotona spp. presence enables us to better understand distribution patterns of 33 hosts implicated in the transmission of Em. The predictive mapping of this Em host enables the

identification of human populations at increased risk of infection, enabling preventativestrategies to be adopted.

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Keywords: *Echinococcus multilocularis*, *Ochotona*, remote sensing, random forests, landscape
 metrics, classification.

39

40 **1. Introduction**

41 Human Alveolar Echinococcosis (HAE), caused by the parasitic tapeworm Echinococcus 42 multilocularis (Em), is an emerging pathogen for which increased prevalence and range 43 expansion is documented in many regions of the northern hemisphere (Eckert, 1996; Eckert et 44 al., 2001). It is a highly pathogenic zoonosis with over 94% mortality in untreated patients ten 45 years after diagnosis (Wang et al., 2010), and is increasingly recognised as a major population 46 health problem (Zhang et al., 2014). The known Em range includes Europe, North America, 47 Japan, the former USSR, Central Asia and China where new foci are being discovered (Wang et al., 2001; Giraudoux et al., 2013a), with prevalence rates of greater than 10% observed in 48 49 Gansu and Sichuan provinces, China (Craig et al., 1992; Li et al., 2010). The spatial 50 distribution of Em is highly variable, with significant regional and local differences in parasite 51 prevalence resulting in patchy distributions generally not reflected in Em and HAE distribution 52 maps (Eckert et al., 2001; Giraudoux et al., 2006; 2013a).

53 The Em transmission cycle is based on the predator-prey relationships between canid 54 definitive hosts such as fox, covote and wolf and small mammal intermediate hosts (Rausch, 55 1995; Eckert et al., 2001). Within a definitive host adult tapeworms produce eggs at regular 56 intervals which are shed in faeces, contaminating the environment (Raoul et al., 2001). The 57 parasite lifecycle then undergoes a free-egg stage, with intermediate hosts infected through oral ingestion of eggs when feeding (Eckert, 1996). The transmission cycle is completed when 58 59 definitive hosts are infected by predating infected intermediate hosts. Em exploits a large 60 number of intermediate host species (>40) (Eckert et al., 2001; Giraudoux et al., 2013b), 61 however the epidemiological importance of these hosts varies (Rausch, 1995).

Domestic dogs can also be infected and, due to their close contact with human populations, are a significant infection risk to humans (Rausch, 1995; Moss *et al.*, 2013; Zhang *et al.*, 2014) via accidental ingestion of Em eggs. Prevalence rates of Em infection in domestic dogs of up to 33% are recorded in Tibetan communities of western Sichuan Province, China (Budke *et al.*, 2005), with Craig *et al.* (2000) and Wang *et al.* (2001) identifying owned dogs as a major transmission source to humans in Gansu Province, and the eastern Tibetan plateau,
China, respectively (Wang *et al.*, 2010).

69 Dog re-infection studies in Sichuan Province, China, suggest that domestic dog 70 populations are quickly re-infected by Em, and may contribute to an active peri-domestic 71 transmission cycle (Giraudoux et al., 2013a; Moss et al., 2013). Wang et al. (2010) also found 72 that Em worm burden in dogs exhibited a statistically significant relationship to maximum 73 burrow densities of a key Em intermediate host, the plateau pika (Ochotona spp.) in the 74 surrounding landscape in Shiqu County, Ganze Tibetan Autonomous Prefecture, China. This 75 study failed to identify significant relationships between dog worm burden and burrow density 76 of another potential Em small mammal intermediate host present in this region, Microtus spp., 77 thus suggesting that the rapid Em re-infection rates in domestic dogs, shown by Moss et al. 78 (2013), is probably linked to surrounding high densities of Ochotona spp.

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80 Small mammal species often exhibit specific preferences for optimal habitats, with 81 species distributions influenced by the locations of these key habitats (Raoul et al., 2008). 82 Small mammal populations are shown to respond to optimal habitat availability, particularly 83 the ratio of optimal habitat to total land area (Giraudoux et al., 2003; Pleydell et al., 2008). 84 Consequently, landscape change is known to affect the population dynamics of wild mammals 85 (Lidicker, 1995), with increases in the optimal habitat proportions correlated with population 86 outbreaks of *Microtus arvalis* and *Arvicola terrestris* in France (Giraudoux *et al.*, 1997), and 87 *M. limnophilus* and *Cricetulus longicaudatus* in south Gansu, China (Giraudoux *et al.*, 1998; 88 Craig et al., 2000). This process is hypothesised to be significant for Em transmission 89 (Giraudoux et al., 1997), so that pathogen transmission may vary through time and space due to landscape modification. Elsewhere in China, small mammal spatial distributions are shown 90 91 to be modified by landscape disturbances such as deforestation in Gansu (Giraudoux et al., 92 1998), afforestation in Ningxia (Raoul et al., 2008), and overgrazing and fencing practices on 93 the Tibetan plateau (Wang et al., 2004; Raoul et al., 2006).

94 Pastureland degradation due to overgrazing has also been linked to increased small 95 mammal densities, for example *Ochotona spp.*, *Microtus spp.*, *Cricetulus kamensis* and

96 Myospalax baileyi (Raoul et al., 2006) on the eastern Tibetan plateau, China, where HAE is

97 endemic (Wang et al., 2004; Li et al., 2010). In Shiqu county, China, grass height was

98 negatively related to Ochotona curzoniae burrow abundance suggesting that overgrazing in this

99 area increased abundance of this species (Wang et al., 2010). With high Ochotona spp.

100 densities significantly associated with infection of domestic dogs (Wang et al., 2010), foxes

and humans (Craig *et al.*, 2000), pastureland degradation resulting from overgrazing could
prove a significant driver of increased human Em incidence in this region.

Previous studies of Em and landscape using remote sensing techniques in southern Gansu Province, China, identified strong links between landscape composition and HAE prevalence (Craig *et al.*, 2000; Giraudoux *et al.*, 2003; Danson *et al.*, 2004). This suggested that grassland and tree/shrub habitats capable of sustaining cyclically high populations of susceptible intermediate hosts were key spatial determinants of Em transmission (Danson *et al.*, 2003), and indicated that landscape composition could provide a useful predictor of Em and HAE (Pleydell *et al.*, 2008; Giraudoux *et al.*, 2013b).

110 On the Tibetan plateau the black-lipped pika or plateau pika (Ochotona curzoniae) is 111 thought to be one of the principal intermediate hosts in the Em transmission cycle (Giraudoux 112 et al., 2006; Zhang et al., 2014). Pika are social mammals that tend to be spatially clumped 113 (Arthur et al., 2008), with average individual home range sizes for Ochotona curzoniae of 114 $1,375 \pm 206 \text{m}^2$ (Smith & Gao, 1991) and population densities ranging from 100 to 400 pikas 115 ha⁻¹ on the Tibetan plateau (Jiapeng et al., 2013). Given the contrast between the biomass of 116 Ochotona spp. (high) to Microtus spp. (low) in Shiqu county (Wang et al., 2010), the role of 117 Ochotona spp. in transmission to dogs may be highly significant (Giraudoux et al., 2013a).

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119 The research presented here builds on this previous work and investigates a critical 120 phase of the Em transmission cycle, where the parasite is carried by small mammal 121 intermediate hosts. Satellite remote sensing and *in-situ* ecological datasets are used to 122 investigate the spatial relationship between Ochotona spp. presence and specific landscape 123 characteristics to identify and better understand these links using random forests. Key 124 landscape variables hypothesised to influence Ochotona spp. presence, and their relative 125 importance, are determined and used to map Ochotona spp. presence over a broader 126 geographical area. The hypotheses addressed are: (1) Ochotona spp. presence is statistically 127 related to key environmental variables which can be used to predict species presence over 128 larger areas; and (2) In the geographical area of interest, Ochotona spp. presence is specifically 129 linked to areas of degraded grassland.

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To identify the key landscape features influencing *Ochotona spp.* presence, random forest (RF) analysis methods are highly appropriate. RF are an ensemble learning technique developed by Breiman (2001) based on a combination of a large set of classification and regression trees. They are well-suited to handling large datasets with correlated predictor variables (Svetnik *et al.*, 2003), handle a variety of data types (Duro *et al.*, 2012), are nonparametric (Strobl *et al.*, 2008), make no assumption of independence concerning the data
being analysed (Perdiguero-Alonso *et al.*, 2008), and are robust to outliers, noise and overfitting (Breiman, 2001). They have been used as analytical tools for a variety of applications
(Svetnik *et al.*, 2003) including remote sensing analysis (Duro *et al.*, 2012; Abdel-Rahman *et al.*, 2013) and parasitological studies (Perdiguero-Alonso *et al.*, 2008).

141 Random forest algorithms employ recursive partitioning to generate multiple decision 142 trees and average individual tree predictions across the entire forest (Duro et al., 2012; Abdel-143 Rahman et al., 2013). Each iteration uses two-thirds of the data to train the RF while the 144 remaining third, the 'out of bag' (OOB) samples, are retained for testing the prediction error of 145 the RF (Duro et al., 2012). The OOB error estimate also generates variable importance 146 measures by comparing increases in OOB error when that variable is randomly permuted while 147 all others are left unchanged, enabling ranking of the importance of individual variables 148 (Abdel-Rahman et al., 2013). The OOB error estimate removes the need for cross-validation 149 via a set-aside test dataset (Perdiguero-Alonso et al., 2008).

150

151 **2.** Materials and methods

152 The research focused on a study area near the town of Tuanji, Shiqu county, Ganze Tibetan 153 Autonomous Prefecture, Sichuan Province, China (Fig 1). This is located on the eastern edge 154 of the Tibetan plateau (Lat 33.04° Lon 97.97°) at altitudes between 4000-4300 metres, and 155 dominated by semi-natural grassland. Although above the tree line, variation in herb and shrub 156 vegetation produces a variety of land cover types. Heavy grazing by yak in this region has 157 resulted in extensive areas of degraded grassland. Within Shiqu county, at least three townships have been found to be local foci for HAE, showing that a transmission cycle is, or has been 158 159 active here (Wang et al., 2001).

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Figure 1. Study site map with numbered survey transects and SRTM DEM (USGS, 2006) site
elevation and UTM WGS84 zone 47N grid displayed. [SINGLE COLUMN FIGURE]



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2.1 Study design 165

166 Fifteen transects of varying length (220-4750m) totaling approximately 35 km and comprising 167 3481 transect points were surveyed in July 2001 (Table 1), with transect routes pre-selected to 168 sample the maximum number of land cover types. At ten meter intervals along the transects 169 small mammal activity indicators were recorded. Visual sightings of small mammals and 170 species-specific indicators including foraging corridors, ground holes, and small mammal 171 faeces, all identifiable to species or genus level (Raoul et al., 2006; Wang et al., 2010), were 172 used as evidence of small mammal presence using methods established by Giraudoux et al. 173 (1998). Transects were mapped using a GPS with an accuracy of approximately 15 m.

174 At this study site the small mammal community predominantly comprised two 175 Ochotona species both known to be Em intermediate hosts, Ochotona curzoniae (black-lipped 176 pika), and Ochotona cansus (Gansu pika), the latter recorded sporadically compared to the 177 former. Due to similarities between the two species resulting in identification difficulties, they 178 were grouped together to form a generic Ochotona spp. group. Microtus irene, M. oeconomus, 179 *M. leucurus* and *Cricetulus kamensis* small mammals were also observed but, given the very

extensive Ochotona spp. colonies in the study area in comparison to the sparse records of these
other species, and the established links between Ochotona spp. and Em infection in dogs
(Wang et al., 2010), our investigation focused exclusively on Ochotona spp.

183 Altitude, slope and aspect values for each transect point were extracted from 90m 184 resolution Shuttle Radar Topographic Mission (SRTM) digital elevation models. A Landsat 185 ETM+ satellite image (3 July 2001) was acquired (path 134 row 37), geometrically corrected, 186 with snow and cloud masks created to exclude these areas of the image from further analysis. 187 ERDAS IMAGINE was used to perform a maximum likelihood supervised classification on 188 the image using nine land cover classes: village, road, long grass, water, short grass, upper 189 Potentilla shrubland, bare ground, degraded grassland, and wet grassland. Classification 190 accuracy assessment was performed using 365 reference points collected from high-resolution 191 imagery of the survey area using established techniques (e.g. Duro et al., 2012). Reference 192 points exhibiting temporal change in land cover type between Landsat ETM+ image and 193 reference high resolution imagery acquisition dates were disregarded to minimise potential 194 error.

195 When investigating the relationships between landscape and Ochotona spp. issues of 196 scale and the spatial arrangement of different land cover class patches within the landscape 197 should be considered (Pleydell et al., 2008; Pleydell & Chrétien, 2010). A common approach is 198 to quantify landscape characteristics around a point of interest using a circular buffer centred at 199 the observation (Pleydell & Chrétien, 2010). However, as the optimal buffer size cannot be 200 known apriori, multiple nested buffers with radius increments between 100m and 500m in 201 100m increments were generated for each transect point, enabling landscape influence over 202 multiple ranges to be investigated. Within each nested buffer, the area of each land cover class 203 was recorded. To minimise collinearity between these nested land cover area measurements 204 (variables calculated using smaller buffers partly measures the same area as the larger buffers), 205 but to retain the nested spatial structure, a new set of variables Z100m, Z200m, Z300m, Z400m 206 and Z500m were created following the methodology of Rhodes et al. (2009) such that:

- 207
- $208 \quad Z100m = X100m.$
- 209 Z200m = X200m X100m.
- 210 Z300m = X300m X200m.
- 211 Z400m = X400m X300m.
- 212 Z500m = X500m X400m.

where X100m,...,X500m are the land cover class coverage data for the 100m,...,500m buffer sizes respectively, and the Z200,...,Z500m provide the difference between the original variables and the variable nested within it (Rhodes *et al.*, 2009).

216 Landscape structure and composition are important determinants of species 217 distributions and population viability (Rhodes et al., 2009), with the amount of suitable habitat 218 present and the level of landscape fragmentation both important factors for biological 219 population abundance and distribution (Fahrig, 2003). Here, the aggregate properties of the 220 spatial organisation of land cover patches within a 500m radius buffer surrounding each 221 transect point are examined using landscape metric methods within FRAGSTATS (McGarigal 222 et al., 2002). Eighteen landscape level metrics were generated (see Table 1). Pairwise 223 correlation was performed between metrics values, with all correlations exhibiting an r² value 224 of <0.5 indicating that the landscape metrics variables were not highly correlated.

225

| Metric Type | Metric | Acronym |
|-----------------------|--|-----------|
| Area and edge metrics | Total Area | TA |
| | Largest Patch Index | LPI |
| | Patch Area Distribution | AREA_AM |
| Shape metrics | Perimeter-Area Ratio Distribution | PARA_AM |
| | Fractal Index Distribution | FRAC_AM |
| | Contiguity Index Distribution | CONTIG_AM |
| Aggregation metrics | Aggregation Index | AI |
| | Patch Cohesion Index | COHESION |
| | Landscape Division Index | DIVISION |
| | Splitting Index | SPLIT |
| | Euclidean Nearest Neighbor Distance Distribution | ENN_AM |
| | Connectance | CONNECT |
| Diversity metrics | Patch Richness | PR |
| | Shannon's Diversity Index | SHDI |
| | Simpson's Diversity Index | SIDI |
| | Shannon's Evenness Index | SHEI |
| | Simpson's Evenness Index | SIEI |

Table 1. Landscape metrics included in the analysis (McGarigal et al., 2002).

227

Random forest (RF) analysis was performed to identify potential causal linkages between *Ochotona spp*. presence and the environmental variables of nested land cover class areas, the landscape metrics, and topographical variables of elevation, slope and aspect (ntrees = 10000, number of variables tried at each split = 21). The OOB data samples generated importance measures for each variable, and tested the prediction error of the generated RF. 233 Random Forest analysis was performed in the R statistical environment using the 234 randomForest package (Liaw & Wiener, 2002). The RF was then used to produce a predicted 235 Ochotona spp. distribution map. A point grid was generated for a 45km x 45km area 236 surrounding the survey transect locations with 30m point spacing. Data values for each 237 explanatory variable included in the RF were calculated for each vector grid point. The RF was 238 applied in a predictive classifier capacity with the vector grid datasets as input variables and 239 predicted Ochotona spp. presence or absence as the output. Predicted values were converted 240 from vector to raster format using ArcMap 10.1.

241

3. Results

243 The overall land cover classification accuracy using 365 reference locations was 83.84%

244 (Table 2). Of the 3481 sample points sampled along 15 transects, *Ochotona spp.* were present

at 1246 points (35.8%). For individual transects the rate of *Ochotona spp*. presence ranged

from 0% (transects 1, 11 and 15) to 88% (transect 2) indicating a patchy distribution across the

study area (Table 3).

| | Referen | ce | | | | | | | | | |
|------------------|---------|-------|-------|-------|-------|------------|--------|-----------|-----------|--------|-----------------|
| Classified | Village | Road | Long | Water | Short | Upper | Bare | Degraded | Wet | Sum of | User's accuracy |
| | | | grass | | grass | potentilla | ground | grassland | grassland | row | (%) |
| | | | | | | shrubland | | | | | |
| Village | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 100.00 |
| Road | 0 | 41 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 44 | 93.18 |
| Long grass | 0 | 0 | 18 | 0 | 0 | 0 | 1 | 0 | 0 | 19 | 94.74 |
| Water | 0 | 1 | 2 | 44 | 1 | 0 | 0 | 1 | 4 | 53 | 83.02 |
| Short grass | 0 | 0 | 0 | 0 | 31 | 2 | 0 | 0 | 0 | 33 | 93.94 |
| Upper potentilla | 0 | 1 | 2 | 0 | 5 | 20 | 0 | 2 | 0 | 30 | 66.67 |
| shrubland | | | | | | | | | | | |
| Bare ground | 0 | 1 | 0 | 0 | 0 | 0 | 44 | 2 | 0 | 47 | 93.62 |
| Degraded | 1 | 2 | 2 | 3 | 7 | 1 | 4 | 45 | 0 | 65 | 69.23 |
| grassland | | | | | | | | | | | |
| Wet grassland | 0 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 41 | 52 | 78.85 |
| | | | | | | | | | | | |
| Sum of column | 23 | 50 | 28 | 50 | 47 | 23 | 49 | 50 | 45 | 365 | |
| Producers | 95.65 | 82.00 | 64.29 | 88.00 | 65.96 | 86.96 | 89.80 | 90.00 | 91.11 | | Overall |
| accuracy (%) | | | | | | | | | | | accuracy = |
| | | | | | | | | | | | 83.84 |

Table 2. Supervised classification confusion matrix and accuracy assessment. Overall Kappa statistic = 0.816

| Transect | Number of | Number of | Number of | Ochotona | Elevation |
|----------|----------------|---------------|---------------|---------------|--------------|
| | survey points | points with | points with | spp. presence | range of |
| | along transect | Ochotona spp. | Ochotona spp. | (%) | transect (m) |
| | | present | absent | | |
| 1 | 276 | 0 | 276 | 0.0 | 4280-4480 |
| 2 | 133 | 117 | 16 | 88.0 | 4290-4334 |
| 3 | 320 | 89 | 231 | 27.8 | 4294-4350 |
| 4 | 94 | 1 | 93 | 1.1 | 4299-4360 |
| 5 | 346 | 28 | 318 | 8.1 | 4287-4350 |
| 6 | 475 | 363 | 112 | 76.4 | 4285-4501 |
| 7 | 274 | 129 | 145 | 47.1 | 4387-4532 |
| 8 | 137 | 61 | 76 | 44.5 | 4309-4484 |
| 9 | 182 | 10 | 172 | 5.5 | 4299-4366 |
| 10 | 424 | 242 | 182 | 57.1 | 4160-4348 |
| 11 | 22 | 0 | 22 | 0.0 | 4160-4160 |
| 12 | 172 | 1 | 171 | 0.6 | 4160-4259 |
| 13 | 339 | 204 | 135 | 60.2 | 4177-4262 |
| 14 | 109 | 1 | 108 | 0.9 | 4182-4300 |
| 15 | 178 | 0 | 178 | 0.0 | 4190-4492 |
| | | | | | |
| Total | 3481 | 1246 | 2235 | 35.8 | 4160-4532 |

249 Table 3. Survey transect *Ochotona spp.* presence and elevation ranges.

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252 RF analysis explained 70.78% of the variance in Ochotona spp. presence or absence. 253 Fig 2 shows the ten environmental variables determined as most important by the RF in 254 relation to Ochotona spp. presence. Aggregation Index (AI) was identified as the single most 255 important variable, however it was the only landscape metric in the top ten ranked variables. 256 Three of the top five variables were degraded grassland (DG), with DG at the 100m buffer size 257 second, at the 300m buffer size fourth, and at the 200m buffer size fifth. Upper Potentilla 258 shrubland (UPS) was also important but at the larger buffer sizes of 400m (third ranked importance), 500m (seventh) and 300m (ninth). Water at 500m was sixth highest ranked, with 259 260 altitude eighth, and short grass (SG) at the 500m buffer tenth.

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Figure 2. Variable importance scores for the top ten variables as identified by the RF, with corresponding % increase in mean square error when that variable is randomly permuted. Percent variance explained = 70.78%, number of trees = 10000, mean square of residuals = 0.07, number of variables tried at each split = 21. AI = Aggregation Index; DG = degraded

266 grassland; UPS = upper *Potentilla* shrubland; SG = short grass. [SINGLE COLUMN FIGURE]





A confusion matrix of the predicted values was generated using the OOB data samples to assess the RF predictive accuracy (Table 4). Results indicate that the RF performed with a high level of accuracy, with a 90.98% accuracy rate. Of the incorrectly predicted samples, the false positives (150) and false negatives (164) were similar in magnitude.

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Table 4. RF confusion matrix of predicted versus observed *Ochotona spp*. presence (1) and absence (0). Total correct = 3167, total incorrect = 314, percentage of survey points predicted correctly = 90.98%

276

| Observed | Predic | Total | | |
|----------|--------|-------|------|--|
| value | value | | | |
| | 0 | 1 | | |
| 0 | 2085 | 150 | 2235 | |
| 1 | 164 | 1082 | 1246 | |
| | | | | |
| Total | 2249 | 1232 | 3481 | |

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279 The map produced (Fig 3) shows the predicted areas of Ochotona spp. presence with

280 patchiness in these areas observed at the local scale. Areas of predicted presence occur across

- the area, but are more extensive to the south, west, and north-west of the original survey
- transects, with sparser areas of predicted presence to the east and north-east.
- 283
- Figure 3. Predicted Ochotona spp. presence (red) or absence (blue) with original survey
- transects overlaid and UTM WGS84 zone 47N grid displayed for context. [SINGLE
- 286 COLUMN FIGURE]



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- 290

4. Discussion

This research examined a critical phase of the *Echinococcus multilocularis* (Em) transmission cycle, and adopted an analytical approach using random forests (RF) to model and predict *Ochotona spp*. presence in relation to landscape characteristics within a highly endemic area of the Tibetan plateau for Em. We found that the environmental variables analysed explained 70.78% of the variance in *Ochotona spp*. presence. It is argued thus that (1) *Ochotona spp*. 297 presence is statistically related to key environmental variables which can be used to predict 298 species presence over large areas; and (2) in the geographical area of interest *Ochotona spp*. 299 presence is specifically linked to areas of degraded grassland.

The application of RF for predictive modelling of Ochotona spp. presence, based on 300 301 landscape characteristics has provided a clearer understanding of the influence of key 302 landscape variables in this region. The environmental variables analysed explained 70.78% of 303 the variance in Ochotona spp. presence, with a 90.98% accuracy rate indicating that the RF 304 methods employed enabled accurate modelling of Ochotona spp. presence. Given these 305 encouraging results, we then generated predictive maps of *Ochotona spp*, presence across a 306 larger spatial extent within the same bio-geographical area to identify potential hot-spots of 307 presence meriting further investigation as reservoir zones of the zoonotic parasite 308 Echinococcus multilocularis.

309 This analysis enabled comparison of the relative importance of the environmental 310 predictors, with the aggregation index (AI) landscape metric ranked with the highest 311 importance. AI is computed where each land cover class is weighted by its area in the 312 landscape, scaled to account for the maximum possible number of like adjacencies given any 313 landscape composition (McGarigal et al., 2002). The interpretation is that buffered areas 314 containing larger aggregations, or clusters of land cover patches of the same type, are of 315 importance in influencing Ochotona spp. presence. However, eight of the ten highest ranked 316 variables are particular land cover class variables suggesting that the presence of specific land 317 cover classes was, with the exception of AI, of greater importance in influencing Ochotona 318 spp. presence than land cover patch spatial arrangement.

319 RF assessment indicated that degraded grassland (DG) at the 100m buffer size was the 320 most important land cover class variable. At the 200m and 300m buffer sizes DG was again the 321 highest ranked land cover variable. Although UPS (400m) and water (500m) were the highest 322 ranked land cover variables at those respective buffer sizes, the ranking of DG as second, 323 fourth and fifth most important variables overall, and highest at the three buffer sizes closest to 324 the survey transect points, indicates that DG could be considered the most important land cover 325 variable of influence. Smith & Gao, (1991) determined that the average home range for 326 Ochotona curzoniae is $1,375 \pm 206 \text{m}^2$, placing the principle area of activity of an individual 327 Ochotona spp. within the 100m buffer area, supporting the RF result that DG at the 100m 328 buffer size is the most important land cover variable influencing Ochotona spp. presence. This 329 reinforces previous studies that have sought to understand the drivers of Ochotona spp. 330 presence in the study region such as Raoul et al. (2006), and visual field observations,

indicating that higher *Ochotona spp*. densities were more commonly present in areas with low vegetation cover. It should be noted, however, that in some areas of degraded grassland where transects were surveyed *Ochotona spp*. were not present. This may be due to patchy local-scale extinctions during *Ochotona spp*. population cycles in this area.

Of particular concern in the study area is the impact of heavy grazing by yak resulting in large areas of degraded grassland. Past studies have shown that land cover changes and grazing practices can increase the likelihood of small mammal population outbreaks that are suggested to play a significant role in Em transmission (Wang *et al.*, 2004). If this heavy grazing results in larger *Ochotona spp*. populations and more frequent population outbreaks due to increased optimal habitat availability, this could potentially contribute to increasing levels of Em transmission, resulting in greater risk to human populations.

342

343 4.1 Conclusions

344 We have used random forests (RF) to successfully model the environmental variables 345 influencing spatial patterns in the presence of the *E. multilocularis* intermediate host Ochotona 346 spp. in western China. The predictive use of random forests to indicate likely areas of 347 Ochotona spp. presence could form a valuable contribution to systematic modelling describing 348 the broader E. multilocularis transmission pathways between Ochotona spp. small mammal 349 intermediate hosts, both sylvatic (fox) and domestic (dog) definitive hosts, and susceptible 350 human populations. Given the relationships established previously by Wang et al. (2010) 351 correlating density of Ochotona spp. burrows with domestic dog infection rates, this 352 methodology could enable identification of domestic dog populations at risk of continual re-353 infection through predation of Ochotona spp. and thus help identify areas of active E. 354 multilocularis transmission. In conjunction with the possibility of applying these techniques 355 over larger geographical regions utilizing the extensive coverage of satellite imagery, such 356 information could facilitate the design of pre-emptive disease control measures including 357 targeted treatment of dogs with antihelminthic drugs to disrupt the Em transmission cycle in 358 that region, thus reducing Em infection risk in local human populations.

359

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